ANNUAL RESEARCH PROGRESS REPORT
FY 2000
Grand Forks Human Nutrition Research Center

ANNUAL RESEARCH PROGRESS REPORT

(FY 2000)

GRAND FORKS HUMAN NUTRITION RESEARCH CENTER

UNITED STATES DEPARTMENT OF AGRICULTURE
AGRICULTURAL RESEARCH SERVICE
NORTHERN PLAINS AREA

GRAND FORKS, NORTH DAKOTA 58202

MINERAL NUTRIENT REQUIREMENTS

MANAGEMENT UNIT

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0149753 Year: 00 Project Number: 5450-51000-012-00 D
Mode Code: 5450-10-00 STP Codes: 5.1.3.3 50% 5.1.3.4 50%
National Program(s): 107 100%

Title: EFFECTS OF COPPER DEPLETION ON CARDIOVASCULAR FUNCTION AND METABOLISM

Period Covered From: 10/99 To: 09/00

Would you like to terminate this Project? N

Will this project terminate within the first 2 months of FY2001? N

Progress and Outcomes:

1. What major problem or issue is being resolved and how are you resolving it?

Diets in the U.S. frequently are low in copper in comparison to dietary standards set by the Food and Nutrition Board of the National Academy of Sciences; these diets resemble the low copper diets that have produced abnormal electrocardiograms, increased cholesterol in blood, impaired metabolism of sugar, and poor control of blood pressure in men and women in controlled experiments. Diets low in copper may contribute to human illness (See Question 2).

The approach to the problem is to identify new biochemistry and physiology of copper with animal experiments to provide functional biomarkers useful in dietary experiments with human volunteers and in community studies. The experiments will identify mechanisms by which adequate dietary copper produces beneficial effects, will identify new effects of human diets low in copper and will contribute to the establishment of national dietary standards. People with biomarkers suggestive of low copper intakes will be supplemented and their responses evaluated.

2. How serious is the problem? Why does it matter?

The major signs of copper deficiency found in depleted men and women and deficient animals resemble the most common characteristics that can predict risk of ischemic heart disease in people. Nearly 80 anatomical, chemical and physiological similarities between animals deficient in copper and people with ischemic heart disease have been identified. It seems likely that the low copper diet common in the U.S. contributes to this disease which is the leading cause of death in the U.S., 480,000 deaths annually. The cost of medical care for this illness is more than $5 billion per year which does not include effects of sorrow, time lost from work or annual cost of prevention (at least $1000 per person). Proper selection of foods may yield diets that meet the

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standards necessary to both illness and expense. This work is relevant to dietitians, food companies, physicians, producers of grain, legumes, nuts and other foods high in copper, public health planners, and teachers of nutrition.

3. How does it relate to the National Program(s) and National Program Component(s)?

The research program fits into the National Program 107, Human Nutrition and Performance Goal 3.1.1. Human nutrition requirements. Emphasis is on biomarkers, mechanisms of action, nutrient interactions and functions as related to healthy hearts and blood vessels to optimize longevity, to decrease disease incidence and to improve productivity. Cooperative studies are in progress with Loma Linda University and the Health Research and Studies Center to evaluate the effects of diets high in complex foods on biomarkers of trace element status and with the Medical College of Ohio to clarify the mechanisms by which copper deficiency alters electrocardiograms.

4. What were the most significant accomplishments this past year?

A. Based on findings from deficiency experiments in animals, it generally is assumed that enzymes dependent on trace elements for activity can be used to assess nutritional status of people. Drs. B. Bruce and G.A. Spiller of the Health Research and Studies Center fed women a diet high in whole and unrefined foods in California. In addition to a favorable effect on blood lipids related to heart disease, we (at the Grand Forks Human Nutrition Research Center) found that activities of enzymes dependent on copper (superoxide dismutase) and selenium (glutathione peroxidase) were affected more by the total diet than by the intake of these elements. These conventional measurements of nutritional status may be useful only in laboratory studies in contrast to field or population studies where diets are quite variable.

B. Evidence that diets low in copper can adversely affect heart function is scattered widely in publications in many scientific fields such as animal husbandry, biochemistry, biology, clinical nutrition, medicine, nutrition, physiology and surgery. Major discoveries about abnormal anatomy, chemistry and physiology of copper deficiency from the Grand Forks Human Nutrition Research Center and elsewhere were presented in historical context. The evidence for low copper status in people was emphasized and characteristics of people

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with heart disease that are found in animals deficient in copper was inferred because approximately one-third of U.S. diets seem to be low in copper. Improving the human intake of copper may decrease expense, morbidity and mortality related to the leading cause of death in the United States.

5. Describe the major accomplishments over the life of the project, including their predicted or actual impact.

A disparity between actual copper intakes and both copper requirements and dietary recommendations has been recognized from the publication by this author of dietary data pooled from 10 research groups in four countries to reveal 60% of daily diets contain less than 1.5 mg of copper and one third contain less than 1 mg. These results refute earlier nutritional beliefs that "adults generally consume 2 to 5 mg of copper daily."

High blood pressure from copper deficiency may be explained partially by the finding of impaired defense against damage by oxygen such as cholesterol oxidation as indicated by findings from copper-deficient animals of an accumulation of a lipid oxidation biomarker called isoprostane and a related impaired ability to relax blood vessels because of the destruction of nitric oxide which is a naturally occurring relaxing agent in blood vessels.

Superoxide dismutases are some of the enzymes that defend us against oxidative damage. The dismutase that depends on adequate dietary copper is impaired in copper deficiency, where, however, genetic control of the dismutase that depends on manganese is improved. This improvement generally is insufficient to eliminate the oxidative damage, and thus pathology caused by an inadequate intake of copper most likely partially involves an impaired defense against damage caused by reactive oxygen species.

All of these findings are related to the mechanisms by which the Western diet gradually damages arteries and hearts leading to ischemic heart disease (see Question 2). Improving diets low in copper by selection of foods high in copper and by decreasing intake of foods low in copper is likely to have great benefits to both health and to decreasing the vast annual expenditures on medical care in middle and old age (see Question 2). Regular consumption of diets adequate in copper may lengthen life and assist in attaining a healthier old age. The ARS Food Pyramid is a useful guide.

6. What do you expect to accomplish, year by year, over the next 3 years?

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During FY 2001 the effect of copper deficiency on the inflammatory process will be evaluated because of the increasingly close association of indices of inflammation such as C-reactive protein to human atherosclerosis and heart disease. The ability of microorganisms such as H. pylori or C. pneumonia to alter cholesterol metabolism and life span of mice will be studied because of the increasingly close association between these organisms and the prevalence of ischemic heart disease. Genetic typing will be used to evaluate data from a human study of the effects of zinc on copper requirements. Several candidate genes have been identified, the most promising of which controls a zinc enzyme related to both heart disease mortality and physical fitness. Zinc intakes are known to greatly affect copper utilization. Other candidate genes are being sought so that they can be evaluated in 2002. A pilot study will be designed using genetic typing so that genetic effects on measurements of trace element status can be sought in appropriate target populations in FY 2003.

7. What science and/or technologies have been transferred and to whom? When is the science and/or technology likely to become available to the end user (industry farmer, other scientists)? What are the constraints, if known, to the adoption and durability of the technology products

The United States Pharmacopeial Convention (USP) provides standards to the Food and Drug Administration in efficacy, quality and safety, etc., of prescription medicines. Dr. Klevay is a member of the USP Advisory Panel of Nutrition and Electrolytes which writes approximately a half dozen nutritional monographs each year. These are incorporated into annually revised volumes published by the USP being immediately available to physicians and pharmacists and other regulatory agencies such as state medical boards.

Information was transferred to the public through articles in the local newspaper (Grand Forks Herald) which also were placed on the Grand Forks Human Nutrition Research Center Home Page; one article was "Low-copper Diets and Osteoporosis: A Link?" by Leslie M. Klevay.

References on the changes in emphasis in copper research over the last 40 years were supplied in response to a request by Dr. Barbara O. Schneeman for the ARS National program Staff.

Bogden, J.D. and Klevay, L.M., editors. Clinical Nutrition of the Essential Trace Elements and Minerals: The Guide for Health Professionals. 1st Edition. The Humana Press, Inc. Totawa, NJ. 2000.

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L.M. Klevay organized the XIII Annual Copper Cardiovascular Cognoscenti dinner at Experimental Biology 2000 Meeting, San Diego, CA.

Several inquiries by individual citizens about hair analysis in nutritional assessment, vegetarian diets, high and low copper foods, environmental cancer, herbal remedies, ideal dietary zinc to copper ratio and selenium as a cancer preventative were answered.

Several inquiries by institutional representatives about hair analysis in nutritional assessment, medical science under dictatorship, the decline in moose populations and microwave effects on food were answered.

8. List your most important publicatons in the popular press (no abstracts) and presentations to nonscientific organizations and articles written about your work (NOTE: this does not replace your peer-reviewed publications which are listed below)

Work from the unit received prominent display in the chapter on copper in Nature's Medicines by G. Maleskey published by Rodale, 1999.

9. Scientific Publications:

01. Bruce, B., Spiller, G.A., Klevay, L.M., Gallagher, S.K. A diet high in whole and unrefined foods favorably alters lipids, antioxidant defenses, and colon function. Journal of the American College of Nutrition. 2000. v. 19. p.61-67.

02. Klevay, L.M. Cardiovascular disease from copper deficiency - a history. Journal of Nutrition. 2000. v. 130. p.489S-492S.

03. Klevay, L.M. Dietary copper and risk of coronary heart disease. American Journal of Clinical Nutrition. 2000. v. 71. p.1213-1214.

04. Klevay, L.M. Trace element and mineral nutrition in ischemic heart disease. Bogden, J.D. and Klevay, L.M., editors. The Humana Press, Inc., Totowa, NJ. Clinical Nutrition of the Essential Trace Elements and Minerals: The Guide for Health Professionals. 2000, p.251-271.

05. Sandstead, H.H., Klevay, L.M. History of nutrition symposium: Trace Element nutrition and human health. Journal of Nutrition. 2000. v. 130. p.483S-484S.

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Publications: (Continued)

06. Spiller, G., Bruce, B., Klevay, L., Gallagher, S. Effects of red wine consumption on plasma antioxidant defenses in generally healthy moderate wine-drinking men. Federation of American Societies for Experimental Biology Journal. 2000. v. 14. p.A520.

07. Klevay, L.M. Biochemistry before you understand the chemistry. In: 32nd Great Lakes 2000 American Chemical Society Regional Meeting Abstracts of Presentations. Fargo, ND. 2000. p. 15.

08. Christopherson, D.M., Shuler, T.R., Klevay, L.M. Hair analysis in environmental assessment. In: 32nd Great Lakes 2000 American Chemical Society Regional Meeting Abstracts of Presentations. Fargo, ND. 2000. p. 18.

09. Klevay, L.M. presented "Medical Science Under Dictatorship-Nuremberg Plus 50" to the Third District Medical Society, Grand Forks, ND.

10. Klevay, L.M. presented "Does Low Dietary Copper Explain the Epidemiologic Link Between Ischemic Heart Disease and Osteoporosis?" to the Sigma Xi, University of North Dakota Chapter, Grand Forks, ND.

11. Klevay, L.M. presented "Does Low Dietary Copper Explain the Epidemiologic Link Between Ischemic Heart Disease and Osteoporosis?" at the University of Arizona, College of Medicine, Tucson, AZ.

12. Klevay, L.M. presented "Biochemistry before you understand the chemistry," at the 32nd Great Lakes Regional Meeting of the American Chemical Society, Fargo, ND, June 4-6, 2000.

13. Christopherson, D.M. presented "Hair analysis environmental assessment" at the 32nd Great Lakes Regional Meeting of the American Chemical Society, Fargo, ND, June 4-6, 2000.

Approved: FORREST H NIELSEN Date: 09/00
Title: CENTER DIRECTOR

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ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0403544 Year: 00 Project Number: 5450-51000-012-01 T
Mode Code: 5450-10-00 STP Codes: 5.1.3.3 50% 5.1.3.4 50%
National Program(s): 107 100%

Title: DETERMINATION OF A NO EFFECT LEVEL FOR COPPER IN DRINKING WATER FOR FUTURE DIETARY SUPPLEMENT STUDY

Period Covered From: 10/99 To: 09/00

Would you like to terminate this Project? N

Will this project terminate within the first 2 months of FY2001? N

Progress and Outcomes:

1. What major problem or issue is being resolved and how are you resolving it?

Diets in the U.S. frequently are low in copper in comparison to dietary standards set by the Food and Nutrition Board of the National Academy of Sciences. This is of concern because low dietary intakes of copper have been associated with the incidence of some chronic diseases such as ischemic heart disease and osteoporosis. Drinking water can sometimes contribute to total copper intake, but this contribution may be limited by regulatory groups attempting to assure quality potable drinking water, in part, by setting low concentration standards for copper.

Human volunteers will be used to identify the chemical forms and doses of copper most useful in dietary supplementation trials and to measure the concentrations of copper in drinking water intolerable to healthy adults.

2. How serious is the problem? Why does it matter?

Some nutritional scientists believe that the best method of defining the nutritional requirement for copper is to conduct dietary supplementation trials. The chemical form of copper in multinutrient supplements recently has been found to be unabsorbable. Supplements with improved absorbability may cause undesirable acute effects such as transient nausea. Moreover, environmental regulatory agencies are reevaluating the quality of drinking water and may decrease the amount of copper because of these undesirable effects that will mandate a change in water purification processes. This research will help establish the safe and tolerable dose of copper as an absorbable supplement and in drinking water. This work is relevant to the Food and Drug Administration, the Environmental Protection Agency, nutritionists, physicians, researchers on heart disease and osteoporosis and various local and state agencies that must respond to federal standards on water quality.

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National Program(s): 107 100%

3. How does it relate to the National Program(s) and National Program Component(s)?

The research fits into the National Program 107 Human Nutrition and the Performance Goal 3.1.1. Human nutrition requirements. Emphasis is on biomarkers, mechanisms of action and environmental factors.

4. What were the most significant accomplishments this past year?

The tolerable concentration of copper in drinking water is unknown. Men and women drank water varying in copper sulfate content and answered questionnaires about symptoms and responses at the Grand Forks Human Nutrition Research Center and at the Universities of Ulster in Northern Ireland and Santiago in Chile. The copper threshold for transient nausea is approximately 4 mg/1. Because this concentration exceeds the U.S. regulatory level of 1.3 mg/1, the Environmental Protection Agency need not change the regulations.

5. Describe the major accomplishments over the life of the project, including their predicted or actual impact.

None. This is a new project.

6. What do you expect to accomplish, year by year, over the next 3 years?

During FY 2001 we will evaluate our data on the response to different amounts of copper sulfate in distilled water and submit it for publication. We will expand the safe and tolerable intake study to include copper sulfate in mineral water. In 2002 we will evaluate and publish the data and terminate the project.

7. What science and/or technologies have been transferred and to whom? When is the science and/or technology likely to become available to the end user (industry farmer, other scientists)? What are the constraints, if known, to the adoption and durability of the technology products

No technology transfer.

8. List your most important publicatons in the popular press (no abstracts) and presentations to nonscientific organizations and articles written about your work (NOTE: this does not replace your peer-reviewed publications which are listed below)

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National Program(s): 107 100%

None.

9. Scientific Publications:

01. Klevay, L.M., Johnson, L.K. Acute effects of copper in drinking water on men and women. Federation of American Societies for Experimental Biology Journal. 2000. v. 14. p.A794 and Journal of Investigative Medicine. 2000. v. 48. p. 193A.

02. Poirer, K.A., Araya, M., Klevay, L.M., Strain, J.J., Nielsen, F.H., Robson, P. , McGoldrick, M.C., Baker, S.R. Determination of a human acute no observed adverse effect level (NOAEL)for copper. The Toxicologist. 2000. v. 54. pp. 73.

03. Klevay, L.M. "Acute effects of copper in drinking water on men and women." Presented at the Experimental Biology Meeting in San Diego, CA, April 15-18, 2000.

Approved: FORREST H NIELSEN Date: 09/00
Title: CENTER DIRECTOR

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Accession: 0400377 Year: 00 Project Number: 5450-51000-020-00 D
Mode Code: 5450-10-00 STP Codes: 5.1.3.2 60% 5.1.3.3 40%
National Program(s): 107 100%

Title: HOMEOSTASIS AND BIOAVAILABILITY OF TRACE ELEMENTS IN HUMANS AND ANIMALS

Period Covered From: 10/99 To: 09/00

Would you like to terminate this Project? N

Will this project terminate within the first 2 months of FY2001? N

Progress and Outcomes:
1. What major problem or issue is being resolved and how are you resolving it?

Our laboratory is attempting to solve two major problems, one associated with manganese nutrition and the other associated with selenium nutrition.
A. Although manganese is an established essential element, its practical nutritional importance has not been ascertained, nor has a safe upper limit of intake been established. Knowledge of safe intakes are needed because of recent speculation that high dietary intakes could have detrimental effects on brain function and behavior. Whether manganese ingested through the diet results in manganese accumulation and predisposition to toxicity, and factors that affect these processes need to be determined.
Approach. Animals and cells in culture will be used to determine factors affecting manganese transport into the cell and/or body, including interactions with other trace elements and nutrients, and whether such interactions may alter uptake and/or retention and promote excessive manganese retention. Similar studies will determine whether proteins involved in iron transport are the mechanism for manganese transport into the cell. Animal studies will determine whether diets low in magnesium and high in manganese result in increased risk of magnesium-deficiency heart disease. Human studies will determine whether diets low in magnesium and moderately high in manganese result in excessive manganese accumulation and pose a practical health risk.
B. The chemical forms of selenium, and the best sources of these forms, which provide optimal health benefits have not been established. Selenium-enriched foods containing specific selenium compounds may be the best way to obtain the maximal health benefits of selenium, especially the ability to prevent colon cancer, increase selenoprotein synthesis and enhance psychological function.
Approach. The efficacy of food sources of selenium for cancer prevention will be determined in rats injected with a carcinogen and fed diets containing selenium supplied as high-selenium meat, wheat or broccoli. To determine the ability of

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high-selenium foods to induce selenoprotein synthesis, animals and cultured cells will be supplied radioactive selenium incorporated into meat, wheat or broccoli. Proteins will be chemically separated and examined for the presence of radioactive selenium. Whether different chemical forms of selenium regulate thioredoxin reductase protein differently will be determined. An inhibitor of thioredoxin reductase will be used to ascertain the possible detrimental health consequences of low activity of this protein. The effect of different food and chemical forms of selenium on the genetic regulation of selenium proteins also will be determined.

2. How serious is the problem? Why does it matter?

Studies of manganese intake and retention are important because excessive intakes of manganese result in toxicity symptoms that resemble Parkinson's disease. Recently, it has been suggested that the amounts of manganese consumed through food may be harmful and that efforts should be made to remove it from the food supply. There is little data too support these assertions, but likewise there is little data to refute them. This research will be of value to physicians, health professionals and regulatory professionals who will make decisions regarding the safety and adequacy of manganese in the North American diet.

The studies of food forms of selenium are important because selenium supplementation is a nutritional issue. Most nutritional professionals agree that the best way to consume nutrients is through the food supply, but presently there are no guidelines regarding the best food forms of selenium. Demonstrating that selenium in a food can produce health benefits similar to dietary supplements will benefit the consumer, as well as producers who may use the information to increase the marketability and/or profitability of their product.

3. How does it relate to the National Program(s) and National Program Component(s)?

These studies relate to the National Program 107, Human Nutrition. This work is related to specific objectives of Bioavailability of Nutrients and Food Components, Health Promoting Properties of Plant and Animal Foods and to Nutrient Requirements.

4. What were the most significant accomplishments this past year?

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A. Concerns about the possibility of either manganese toxicity or deficiency in free-living humans consuming mixed western diets, and the paucity of well-controlled human studies addressing these questions, were the impetus for a comprehensive human study that examined health effects of consuming diets made as low or high in manganese as practically possible. Human volunteers were fed less than 1 or 20 milligrams of manganese a day for sixty days, and clinical, neurological and physiological variables were closely monitored. Manganese fed at the maximum or lowest amount practical in a mixed Western diet affected manganese retention but did not have any deleterious health consequences to healthy young women. The results indicate, that in the absence of exacerbating circumstances and/or dietary interactions, there is no practical reason to fear for human manganese deficiency or toxicity.
B. Because of the reports of the anti-cancer properties of selenium, many persons would like to consume more selenium through their diet, but different foods contain different forms of selenium, and the optimal food form(s) of selenium have not been identified. We developed a method to grow broccoli that contains over one thousand times the normal amount of selenium, and fed this broccoli to rats injected with a colon carcinogen. Rats that consumed selenium from high-selenium broccoli had an almost 50% reduction in the incidence of colon cancer. Continued research in this area will help elucidate optimal food sources of selenium for maximal health benefits.

5. Describe the major accomplishments over the life of the project, including their predicted or actual impact.

Demonstrated that supplemental selenium improves the mood of healthy young men. This effect was seen in selenium-adequate and selenium-deficient men. This research adds to the increasing number of studies showing that supplemental intakes of selenium improve health and well-being.
Demonstrated in rats that selenium from high-selenium broccoli was retained and distributed differently than selenium from salts such as selenite and selenate. The difference in the distribution of selenium allows selenium from broccoli to be less toxic to rats than other forms of selenium, and also to have greater anti-cancer activity. This research demonstrates that all selenium-containing foods are not equal, and that health professionals need to consider the source of selenium before recommending increased intakes.

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Developed a process to label broccoli with stable isotopes of selenium, and demonstrated in healthy young men that selenium from broccoli did not accumulate to the extent that selenium from salts did. This study demonstrated in humans that selenium from broccoli is less toxic than inorganic chemical forms of selenium.

Described, by using cultured cells, how the cell accumulates manganese and demonstrated that manganese is quickly moved in a direction that results in excretion into the gut, whereas movement in the direction of absorption is slow. This finding shows that animals have developed complex mechanisms to keep manganese from accumulating in the body, and that in the absence of exacerbating factors and interactions, manganese toxicity is probably not of practical concern.

Demonstrated in humans that very little manganese is absorbed, and that absorption is strongly associated with the iron status of an individual. This finding demonstrates that interactions of other nutrients with manganese may potentiate manganese absorption. Thus future studies of possible manganese toxicity should concentrate on possible adverse interactions.

Demonstrated that magnesium-deficient pigs that are fed moderately high amounts of manganese are at an increased risk of sudden death by heart disease. This finding also demonstrates that interactions of other nutrients with manganese may potentiate manganese absorption/retention, and that studies of possible manganese toxicity should concentrate on adverse interactions.

6. What do you expect to accomplish, year by year, over the next 3 years?

Year 1. The mechanism through which pigs fed high dietary manganese and low dietary magnesium become more susceptible to ischemic heart disease will be determined. Tissue histology methods will be used to determine whether mitochondrial structure is altered. Biochemical studies will determine whether calcium pump function is impaired. Electrophysiological measures of the heart will determine whether the heart rhythm is altered.

The regulation and possible anti-cancer functions of thioredoxin reductase, a selenium protein, will be elucidated in laboratory animals. An inhibitor of thioredoxin reductase will be used to knock out the activity of this enzyme in one set of animals, which will be compared to another set of animals with normal enzyme function in their susceptibility to chemically-induced cancer.

Year 2. Studies to identify the basis for the association

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between heart disease, low dietary magnesium and high dietary manganese will continue. They will concentrate on the impairment of the calcium pump as a possible cause of altered mitochondrial function. Cellular methods will be used to measure the movement of calcium inside cells that are stressed by high manganese and low magnesium. Animal studies will also determine how different food forms of selenium alter the production of selenoproteins.

Year 3. After careful examination of the results of animal studies, a human study will be set up to examine possible adverse interactions between low magnesium and high manganese. Subjects will have extensive medical supervision and heart rate and rhythm will also be extensively monitored.

Animal studies will be conducted to determine the bioavailability of manganese from mutant peas that accumulate abnormally high amounts of manganese. If the peas prove suitable as a source of manganese, further studies will determine whether vegetarian diets are at risk of providing too much dietary manganese.

7. What science and/or technologies have been transferred and to whom? When is the science and/or technology likely to become available to the end user (industry farmer, other scientists)? What are the constraints, if known, to the adoption and durability of the technology products

Research was transferred to customers by a variety of mechanisms including:
Seminars presented to visiting dietitians at the Grand Forks Human Nutrition Research Center (2 occasions).
Seminar presented to visiting farm-exchange students at the Grand Forks Human Nutrition Research Center.
Co-taught the class 'Vitamins and Minerals' in the Departments of Animal Science and Nutrition at North Dakota State University.
Presented nutritional information to University of North Dakota medical students during a two-hour question and answer session.
Interviewed by the Grand Forks Herald on high-selenium broccoli and colon cancer research.
Advised the National Council for Reliable Health Information on how many different biochemical forms of selenium occur in foods.
Information transferred to the public through articles in the Grand Forks Herald, "Do you sell a commodity or food?," and "Genetically modified foods - Agriculture's bright future or dark nightmare."

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8. List your most important publicatons in the popular press (no abstracts) and presentations to nonscientific organizations and articles written about your work (NOTE: this does not replace your peer-reviewed publications which are listed below)

Article about high-selenium broccoli appeared in June, 2000 issue of "Agricultural Research" magazine.
Interviewed (June 2000) by Voice of America radio program concerning the cancer-preventive properties of selenium from broccoli.
Interviewed (June 2000) by USDA radio program concerning the cancer-preventive properties of selenium from broccoli.
Work with high-selenium broccoli covered by Grand Forks Herald (July, 2000).

9. Scientific Publications:

01. Finley, J.W., Davis, C.D. Manganese deficiency and toxicity: Are high or low dietary amounts of manganese cause for concern? Biofactors. 1999. v. 10. p. 15-24.

02. Finley, J.W., Duffield, A., Ha, P., Vanderpool, R.A., Thomson, C.D. Selenium supplementation affects the retention of stable isotopes of selenium in human subjects consuming diets low in selenium. British Journal of Nutrition. 1999. v. 82. p. 357-360.

03. Finley, J., Davis, C., Penland, J. Interactive effects of dietary fat type and manganese concentration in healthy young women. FASEB Journal. 2000. v. 14. p. A297.

04. Finley, J., Davis, C., Gregoire, B. Selenium from high-selenium broccoli does not induce glutathione peroxidase (GSH-PX) MRNA production as well as selenite, but is more effective in inhibiting colon carcinogenesis. FASEB Journal. 2000. v. 14. p. A169.

05. Miller, K.B., Caton, J.S., Finley, J.W. High dietary manganese lowers heart magnesium in pigs fed a low magnesium diet. In: 32nd Great Lakes 2000 American Chemical Society Regional Meeting Abstracts of Presentations. Fargo, ND. 2000. p. 15.

06. Gregoire, B.R., Finley, J.W., Davis, C.D., Hintze, K.J. Selenium from high-selenium broccoli is protective against colon. In: 32nd Great Lakes 2000 American Chemical Society Regional Meeting Abstracts of Presentations. Fargo, ND. 2000. p. 16.

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0400377 Year: 00 Project Number: 5450-51000-020-00 D
Mode Code: 5450-10-00 STP Codes: 5.1.3.2 60% 5.1.3.3 40%
National Program(s): 107 100%

Publications: (Continued)

07. Finley, J.W."Selenium from high-selenium broccoli is protective against colon cancer" presented at the Canadian Association of Animal Science Meeting, Winnipeg, Canada, July 19, 2000.

08. Finley, J. "Interactive effects of dietary fat type and manganese concentration in healthy young women" presented at the Experimental Biology 2000 Meeting, San Diego, Ca, April 15-18, 2000.

09. Finley, J. "Selenium from high-selenium broccoli does not induce glutathione peroxidase (GSH-PX) MRNA production as well as selenite, but is more effective in inhibiting colon carcinogenesis" presented at the Experimental Biology 2000 Meeting, San Diego, CA, April 15-18, 2000.

10. Finley, J.W. "Selenium from high-selenium broccoli is protective against colon cancer" presented at the 32nd Great Lakes Regional Meeting of the American Chemical Society, Fargo, ND, June 4-6, 2000.

11. Finley, J.W. Seminar, "Selenium and colon cancer: The food you eat does make a difference" presented at the Children's Nutrition Research Center, Houston, TX, November 18, 1999.

12. Finley, J.W. Seminar, "Selenium biochemistry and the immune system: excitement of recent findings" presented to Anatomy Department (University of North Dakota) and Department of Nutrition (North Dakota State University), January 31, 2000.

Approved: FORREST H NIELSEN Date: 09/00
Title: CENTER DIRECTOR

UNOFFICIAL

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0401877 Year: 00 Project Number: 5450-51000-020-01 S
Mode Code: 5450-10-00 STP Codes: 5.1.3.2 60% 5.1.3.3 40%
National Program(s): 107 100%

Title: SELENIUM IN NORTH DAKOTA BEEF

Period Covered From: 10/99 To: 09/00

Would you like to terminate this Project? N

Will this project terminate within the first 2 months of FY2001? N

Progress and Outcomes:

1. What major problem or issue is being resolved and how are you resolving it?

Selenium-enriched foods may be the best way to increase dietary selenium to obtain its health benefits, but whether the benefits vary with different foods with different chemical forms of selenium, and sources of foods containing enriched amounts of the most effective form of selenium, have not been determined. Beef is the single greatest source of dietary selenium for North Americans. North Dakota has soils high in selenium and may produce beef enriched in selenium. The purpose of this project is to determine whether beef grown in North Dakota is enhanced in selenium and whether there are health benefits associated with consuming selenium in this form. The approach to resolving the problem is a series of field and laboratory studies that determine the selenium content of North Dakota beef, whether this selenium content can be enhanced by feeding high-selenium agricultural products, and whether the beef produced in this manner has health benefits.

2. How serious is the problem? Why does it matter?

The provision of luxuriant amounts of selenium is an important issue because selenium deficiency results in a fatal disease in China, and selenium supplementation has been demonstrated to markedly reduce the incidence of prostate (as well as lung and colo-rectal) cancer in the United States. Findings from these studies will be of value to health and nutritional professionals that are in a position to give advice regarding the best dietary sources of essential nutrients. The results of these studies also may benefit cattle producers who may be able to use the information to enhance the marketability/profitability of their product.

3. How does it relate to the National Program(s) and National Program Component(s)?

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0401877 Year: 00 Project Number: 5450-51000-020-01 S
Mode Code: 5450-10-00 STP Codes: 5.1.3.2 60% 5.1.3.3 40%
National Program(s): 107 100%

This research relates to National Program 107, Human Nutrition, and specific objectives of Health Promoting Properties of Plant and Animal Foods and Bioavailability of Nutrients and Food Components.

4. What were the most significant accomplishments this past year?

A. Because of the national and international interest in increasing selenium in human diets, and because beef is a major source of selenium, a study was conducted to determine how much selenium accumulates in the muscle of beef cattle fed high-selenium feedstuffs. In collaboration with Dr. J. Caton at North Dakota State University, steers were placed in a feedlot and fed hay and grain that had either very high or normal concentrations of selenium. Beef cattle accumulated selenium in muscle many fold above normal when fed high selenium feed, and showed no signs of selenium toxicity during the feeding period. The ultimate benefit of this information will be to enhance the image of beef as an excellent source of selenium which has anti-cancer properties.

B. None.

C. Beef production is one of the most troubled sectors of the farm economy. This has been because demand for beef has steadily fallen, in part because of a negative image of the nutritional value of beef, while production prices have risen steadily. Showing the nutritional benefits of beef will improve the image of beef. The findings from this research could conceivably lead to a specialty niche market for high-selenium, nutrient-enhanced beef, thus providing a source of added income for many small producers.

5. Describe the major accomplishments over the life of the project, including their predicted or actual impact.

A study was completed that measured the selenium in beef from various areas in North Dakota, and demonstrated that some areas of the state produced beef that was 3-5 fold greater in selenium content than the national average. The selenium content of the beef was significantly associated with soil and forage selenium concentrations collected from the same locations. These results indicate some North Dakota beef is an excellent source of selenium which has anti-cancer properties.

6. What do you expect to accomplish, year by year, over the next 3 years?

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Report of Progress (AD-421)

Accession: 0401877 Year: 00 Project Number: 5450-51000-020-01 S
Mode Code: 5450-10-00 STP Codes: 5.1.3.2 60% 5.1.3.3 40%
National Program(s): 107 100%

In the years 2001 and 2002 the effectiveness of high-selenium beef in suppressing the incidence of colon cancer will be demonstrated. The project will end in 2002.

7. What science and/or technologies have been transferred and to whom? When is the science and/or technology likely to become available to the end user (industry farmer, other scientists)? What are the constraints, if known, to the adoption and durability of the technology products

The science of these studies has been transferred to the public by:
Presentation of research results at the North Dakota State Extension Service Beef field day (March 2000).
Presentation of results to the North Dakota Beef Commission and to individual members of the North Dakota Beef Commission.

8. List your most important publicatons in the popular press (no abstracts) and presentations to nonscientific organizations and articles written about your work (NOTE: this does not replace your peer-reviewed publications which are listed below)

None

9. Scientific Publications:

01. Hintze, K.J., Lardy, G.P., Marchello, M.J., Finley, J.W. Selenium in beef grass and soil from various regions of North Dakota. In: 32nd Great Lakes 2000 American Chemical Society Regional Meeting Abstracts of Presentations. Fargo, ND. 2000. p. 16.

02. Hintze, K.J. presented "Selenium in beef grass and soil from various regions of North Dakota" at the 32nd Great Lakes Regional Meeting of the American Chemical Society, Fargo, ND, June 4-6, 2000.

Approved: FORREST H NIELSEN Date: 09/00
Title: CENTER DIRECTOR

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ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0402745 Year: 00 Project Number: 5450-51000-020-02 T
Mode Code: 5450-10-00 STP Codes: 5.1.3.2 60% 5.1.3.3 40%
National Program(s): 107 100%

Title: PRODUCTION OF HIGH SELENIUM BEEF

Period Covered From: 10/99 To: 09/00

Would you like to terminate this Project? Y

Will this project terminate within the first 2 months of FY2001?

Progress and Outcomes:

1. What major problem or issue is being resolved and how are you resolving it?

Selenium-enriched foods may be the best way to increase dietary selenium to obtain its health benefits, but whether the benefits vary with different foods with different chemical forms of selenium, and sources of foods containing enriched amounts of the most effective form of selenium, have not been determined. Beef is the single greatest source of dietary selenium for North Americans. North Dakota has soils high in selenium and may produce beef enriched in selenium. This project will determine whether beef grown in North Dakota is a rich source of selenium and whether there are health benefits associated with consuming selenium in the form of beef. The approach to solving the problem is a series of field and laboratory studies that are determining the selenium content of North Dakota beef, whether this selenium content can be enhanced by feeding high-selenium agricultural products, and whether the beef produced in this manner has health benefits.

2. How serious is the problem? Why does it matter?

The provision of luxuriant amounts of selenium to people through foods is an important issue because selenium deficiency results in a fatal disease in China, and selenium supplementation has been demonstrated to markedly reduce the incidence of prostate (as well as lung and colo-rectal) cancer in the United States. Findings from these studies will be of value to health and nutritional professionals that are in a position to give advice regarding the best dietary sources of essential nutrients. The results of these studies also may benefit cattle producers who may be able to use the information to enhance the marketability/profitability of their product.

3. How does it relate to the National Program(s) and National Program Component(s)?

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Report of Progress (AD-421)

Accession: 0402745 Year: 00 Project Number: 5450-51000-020-02 T
Mode Code: 5450-10-00 STP Codes: 5.1.3.2 60% 5.1.3.3 40%
National Program(s): 107 100%

This research relates to National Program 107, Human Nutrition, and specific objectives of Health Promoting Properties of Plant and Animal Foods and Bioavailability of Nutrients and Food Components.

4. What were the most significant accomplishments this past year?

A. Because of the national and international interest in increasing selenium in human diets, and because beef is a major source of selenium, a study was conducted to determine how much selenium accumulates in the muscle of beef cattle fed high-selenium feedstuffs. In collaboration with Dr. J. Caton at North Dakota State University, steers were placed in a feedlot and fed hay and grain that had either very high or normal concentrations of selenium. Beef cattle accumulated selenium in muscle many-fold above normal when fed high selenium feed, and showed no signs of selenium toxicity during the feeding period. The ultimate benefit of this information will be to enhance the image of beef as an excellent source of selenium which has anti-cancer properties.

B. None.

C. Beef production is one of the most troubled sectors of the farm economy. This has been because demand for beef has steadily fallen, in part because of a negative image of the nutritional value of beef, while production prices have risen steadily. Showing the nutritional benefits of beef will improve the image of beef. The findings from this research could conceivably lead to a specialty niche market for high-selenium, nutrient-enhanced beef, thus providing a source of added income for many small producers.

5. Describe the major accomplishments over the life of the project, including their predicted or actual impact.

A study was completed that measured the selenium in beef from various areas in North Dakota, and demonstrated that some areas of the state produced beef that was 3-5 fold greater in selenium content than the national average. The selenium content of the beef was significantly associated with soil and forage selenium concentrations collected from the same locations. These results demonstrate that some North Dakota beef is an excellent source of selenium which has anti-cancer properties.

6. What do you expect to accomplish, year by year, over the next 3 years?

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Accession: 0402745 Year: 00 Project Number: 5450-51000-020-02 T
Mode Code: 5450-10-00 STP Codes: 5.1.3.2 60% 5.1.3.3 40%
National Program(s): 107 100%

The agreement with the North Dakota Agricultural Products Utilization Commission (APUC) was completed in November, 1999. A final report has been mailed to the ND APUC. Objectives similar to this project are currently being funded by the North Dakota Beef Commission.

7. What science and/or technologies have been transferred and to whom? When is the science and/or technology likely to become available to the end user (industry farmer, other scientists)? What are the constraints, if known, to the adoption and durability of the technology products

The science of these studies has been transferred to the public by:
Final report mailed to the North Dakota APUC.

8. List your most important publicatons in the popular press (no abstracts) and presentations to nonscientific organizations and articles written about your work (NOTE: this does not replace your peer-reviewed publications which are listed below)

None

9. Scientific Publications:
01. Hintz, K.J. "Factors that influence selenium content of beef" presented at the Canadian Association of Animal Science Meeting, Winnipeg, Canada, July 15-19. 2000.

Approved: FORREST H NIELSEN Date: 09/00
Title: CENTER DIRECTOR

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ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0402749 Year: 00 Project Number: 5450-51000-020-03 T
Mode Code: 5450-10-00 STP Codes: 5.1.3.2 60% 5.1.3.3 40%
National Program(s): 107 100%

Title: HIGH SELENIUM BEEF PRODUCED IN NORTH DAKOTA

Period Covered From: 10/99 To: 09/00

Would you like to terminate this Project? N

Will this project terminate within the first 2 months of FY2001? N

Progress and Outcomes:

1. What major problem or issue is being resolved and how are you resolving it?

Selenium-enriched foods may be the best way to increase dietary selenium to obtain its health benefits, but whether the benefits vary with different foods with different chemical forms of selenium, and sources of foods containing enriched amounts of the most effective form of selenium, have not been determined. Beef is the single greatest source of dietary selenium for North Americans. North Dakota has soils high in selenium and may produce beef enriched in selenium. The purpose of this project is to determine whether beef grown in North Dakota is a rich source of selenium and whether there are health benefits associated with consuming selenium in the form of beef. The approach to solving the problem is a series of field and laboratory studies that are determining the selenium content of North Dakota beef, whether this selenium content can be enhanced by feeding high-selenium agricultural products, and whether the beef produced in this manner has health benefits.

2. How serious is the problem? Why does it matter?

The provision of luxuriant amounts of selenium to people through foods is an important issue because selenium deficiency results in a fatal disease in China, and selenium supplementation has been demonstrated to markedly reduce the incidence of prostate (as well as lung and colo-rectal) cancer in the United States. Findings from these studies will be of value to health and nutritional professionals that are in a position to give advice regarding the best dietary sources of essential nutrients. The results of these studies also may benefit cattle producers who may be able to use the information to enhance the marketability/profitability of their product.

3. How does it relate to the National Program(s) and National Program Component(s)?

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0402749 Year: 00 Project Number: 5450-51000-020-03 T
Mode Code: 5450-10-00 STP Codes: 5.1.3.2 60% 5.1.3.3 40%
National Program(s): 107 100%

This research relates to National Program 107, Human Nutrition, and specific objectives of Health Promoting Properties of Plant and Animal Foods and Bioavailability of Nutrients and Food Components.

4. What were the most significant accomplishments this past year?

A. Because of the national and international interest in increasing selenium in human diets, and because beef is a major source of selenium, a study was conducted to determine how much selenium accumulates in the muscle of beef cattle fed high-selenium feedstuffs. In collaboration with Dr. J. Caton at North Dakota State University, steers were placed in a feedlot and fed hay and grain that had either very high or normal concentrations of selenium. Beef accumulated selenium in muscle many-fold above normal when fed high selenium feed, and showed no signs of selenium toxicity during the feeding period. The ultimate benefit of this information will be to enhance the image of beef as an excellent source of selenium which has anti-cancer properties.

B. None

C. Beef production is one of the most troubled sectors of the farm economy. This has been because demand for beef has steadily fallen, in part because of a negative image of the nutritional value of beef, while production prices have risen steadily. Showing the nutritional benefits of beef will improve the image of beef. The findings from this research could conceivably lead to a specialty niche market for high-selenium, nutrient-enhanced beef, thus providing a source of added income for many small producers.

5. Describe the major accomplishments over the life of the project, including their predicted or actual impact.

A study was completed that measured the selenium in beef from various areas in North Dakota, and demonstrated that some areas of the state produced beef that was 3-5 fold greater in selenium content than the national average. The selenium content of the beef was significantly associated with soil and forage selenium concentrations collected from the same locations. Some North Dakota beef is an excellent source of selenium which has anti-cancer properties.

6. What do you expect to accomplish, year by year, over the next 3 years?

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Accession: 0402749 Year: 00 Project Number: 5450-51000-020-03 T
Mode Code: 5450-10-00 STP Codes: 5.1.3.2 60% 5.1.3.3 40%
National Program(s): 107 100%

In the years 2001 and 2002, the effectiveness of high-selenium beef in suppressing the incidence of colon cancer will be determined. The project will end in 2002.

7. What science and/or technologies have been transferred and to whom? When is the science and/or technology likely to become available to the end user (industry farmer, other scientists)? What are the constraints, if known, to the adoption and durability of the technology products

The science of these studies has been transferred to the public by:
Presentation of research results at the North Dakota State Extension Service Beef field day (March 2000).
Presentation of results to the North Dakota Beef Commission and to individual members of the North Dakota Beef Commission.

8. List your most important publicatons in the popular press (no abstracts) and presentations to nonscientific organizations and articles written about your work (NOTE: this does not replace your peer-reviewed publications which are listed below)

None

9. Scientific Publications:

Approved: FORREST H NIELSEN Date: 09/00
Title: CENTER DIRECTOR

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0402795 Year: 00 Project Number: 5450-51000-020-04 T
Mode Code: 5450-10-00 STP Codes: 5.1.3.2 100% %
National Program(s): 107 100%

Title: HEALTH BENEFITS OF FOOD FORMS OF SELENIUM

Period Covered From: 10/99 To: 09/00

Would you like to terminate this Project? N

Will this project terminate within the first 2 months of FY2001? N

Progress and Outcomes:

1. What major problem or issue is being resolved and how are you resolving it?

Selenium-enriched foods may be the best way to increase dietary selenium to obtain its health benefits, but whether the benefits vary with different foods with different chemical forms of selenium, and sources of foods containing enriched amounts of the most effective form of selenium, have not been determined. Wheat and wheat products are major sources of dietary selenium for North Americans. Additionally, for many people, fortification of grain products made from grains has been a primary means of increasing their trace mineral intake. Much wheat grown in North and South Dakota is naturally high in selenium, and a natural way to fortify grain products would be to use their high-selenium grains as an ingredient. Prior to doing this, however, it is important to know whether selenium from wheat confers any of the health benefits that have been associated with selenium supplementation. Thus, this project will study the metabolism and cancer preventive properties of selenium from high-selenium wheat products.

Approach. High-selenium wheat will be purchased from an area known to produce high-selenium agricultural crops and will be milled into a suitable wheat product. Cancer incidence and selenium metabolism will be studied in animals fed diets containing the high-selenium wheat product.

2. How serious is the problem? Why does it matter?

The provision of luxuriant amounts of selenium to people through foods is an important issue because selenium deficiency results in a fatal disease in China, and selenium supplementation has been demonstrated to markedly reduce the incidence of prostate (as well as lung and colo-rectal) cancer in the United States. Findings from this research may result in a dietary approach to reducing the incidence of certain forms of cancer in the United States. Improving the marketability/profitability of wheat because of its health-

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Report of Progress (AD-421)

Accession: 0402795 Year: 00 Project Number: 5450-51000-020-04 T
Mode Code: 5450-10-00 STP Codes: 5.1.3.2 100% %
National Program(s): 107 100%

promoting properties is of importance to wheat producers.

3. How does it relate to the National Program(s) and National Program Component(s)?

This research relates to National Program 107, Human Nutrition, and specific objectives of Health Promoting Properties of Plant and Animal Foods and Bioavailability of Nutrients and Food Components.

4. What were the most significant accomplishments this past year?

A. Because determining the possible health benefits of selenium in wheat could be of great benefit to both wheat producers and consumers, a study was conducted to determine the anti-cancer properties of selenium from wheat. High-selenium wheat was milled into a wheat product and then used as a selenium source for rats injected with a carcinogen. Rats fed selenium from wheat had fewer tumors than rats fed no extra selenium. This finding demonstrates that selenium from wheat provides protection against chemically-induced colon tumors in animals, and strengthens the argument for using high-selenium wheat from North Dakota and South Dakota as a source of selenium enrichment of wheat products.

B. None.

C. If companies that produce grain foods from wheat can be persuaded to use high-selenium wheat to fortify their products with selenium, then there will be a sustainable demand for high-selenium wheat that could be sold for a premium. This will directly benefit small wheat producers in North Dakota and South Dakota.

5. Describe the major accomplishments over the life of the project, including their predicted or actual impact.

No other work has been accomplished over the life of this project.

6. What do you expect to accomplish, year by year, over the next 3 years?

Year 1: The ability of selenium from wheat to decrease colon cancer in experimental animals will be confirmed. Additional experimentation will determine the range of intakes that provides protection against colon cancer.

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National Program(s): 107 100%

Year 2: Studies will examine the basic metabolism of selenium from wheat and will determine whether selenium from wheat is effective in maintaining optimal activity of all selenoproteins. Funds are not currently available to maintain this project for more than two years.

7. What science and/or technologies have been transferred and to whom? When is the science and/or technology likely to become available to the end user (industry farmer, other scientists)? What are the constraints, if known, to the adoption and durability of the technology products

The science of these studies has been transferred to the public by informal discussion of the results with personnel from General Mills, Inc., and it has been formally transferred by means of a CRADA with General Mills, Inc.

8. List your most important publicatons in the popular press (no abstracts) and presentations to nonscientific organizations and articles written about your work (NOTE: this does not replace your peer-reviewed publications which are listed below)

None

9. Scientific Publications:

Approved: FORREST H NIELSEN Date: 09/00
Title: CENTER DIRECTOR

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0400398 Year: 00 Project Number: 5450-51000-021-00 D
Mode Code: 5450-10-00 STP Codes: 5.1.3.2 60% 5.1.3.3 40%
National Program(s): 107 100%

Title: BIOAVAILABILITY OF TRACE ELEMENTS, ESPECIALLY IRON FROM FOOD, & ITS INFLUENCE ON NUTRITURE & FUNCTION

Period Covered From: 10/99 To: 09/00

Would you like to terminate this Project? N

Will this project terminate within the first 2 months of FY2001? N

Progress and Outcomes:

1. What major problem or issue is being resolved and how are you resolving it?

Concerns about the adverse effects of iron deficiency on cognition and physical performance as well as concerns about high iron stores increasing the risk of chronic diseases (such as cancer and heart disease)emphasize the need to determine how dietary iron bioavailability should alter dietary advice for the public.

Both short-term iron absorption and longer-term iron status are being measured in humans consuming controlled diets for several weeks to help determine the true importance of dietary iron bioavailability, and the related impact on practical dietary choices such as consuming less meat, more beans and whole grains, or more tea. The bioavailability of other mineral nutrients, such as calcium, copper, and zinc, may also be affected by such dietary choices, and information on bioavailability of these other mineral nutrients can often be efficiently derived from the same human studies. The iron research will determine the practical importance of dietary iron bioavailability, and how extensively biological adaptation modifies it.

2. How serious is the problem? Why does it matter?

Iron nutrition is a double-edged sword. While adequate iron nutriture in infants, children, and childbearing age women is essential for cognitive development and work capacity, excessive body iron in men and postmenopausal women is hypothesized to increase oxidative stress and the risk of chronic diseases such as heart disease and cancer. Research on dietary iron bioavailability provides information for policy decisions about recommended dietary allowances, food enrichment and fortification standards, and dietary guidelines for the public.

3. How does it relate to the National Program(s) and National Program Component(s)?

ANNUAL RESEARCH PROGRESS REPORT
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Accession: 0400398 Year: 00 Project Number: 5450-51000-021-00 D
Mode Code: 5450-10-00 STP Codes: 5.1.3.2 60% 5.1.3.3 40%
National Program(s): 107 100%

The research fits into the National Program 107, Human Nutrition (100%), and is directly related to Human Nutrition Performance Goal 3.1.1 Human Nutrition Requirements and 3.1.3 Nutritious Plant and Animal Products, concerning the priority objective: Bioavailability of Nutrients and Food Components.

4. What were the most significant accomplishments this past year?

A. Recent concerns about possible excessive body iron accumulation by men consuming Western diets have heightened the need to understand how the human body controls the absorption of heme iron (40% of the iron in meat, poultry, and fish), in comparison to nonheme iron (from various foods and fortified products). We developed a new method to measure in humans, for the first time, the initial intestinal uptake of the two forms of iron, as well as the subsequent transfer to the body. The initial uptake and absorption of heme iron (36% and 15% of dose, respectively) were much higher than for nonheme iron (11% and 7% of dose, respectively), but initial nonheme iron uptake, and consequently its absorption, were more closely related to a person's iron status. The results, indicating that initial iron uptake is the major site of absorptive control, and that this initial entry is more controlled for nonheme than for heme iron, impact the development of nutritional guidelines to the public and food industry to help prevent iron deficiency as well as iron overload.

B. Vegetarian diets have been associated with lower serum copper, and have been proposed as potentially useful in the treatment of Wilson's disease, a disorder of body copper accumulation. We used stable copper isotope to measure copper absorption by women who ate controlled lactoovovegetarian and omnivorous diets for 8 wk each. Although the efficiency of copper absorption from the vegetarian diet was less (33%) than from the omnivorous diet (42%), because the vegetarian diet contained 50% more copper, more total copper was absorbed from the vegetarian (0.48 mg/d), than from the omnivorous diet (0.40 mg/d). Together with previous research, these results suggest that vegetarian diets reduce plasma copper slightly, but this reduction is not caused by reduced dietary copper absorption, and that vegetarian diets may not be effective in treating copper accumulation disorders.

To help assess the nutritional impact of encouraging the use of brown, versus well-milled rice in the Phillipines, we evaluated the effect of milling on the nutritional bioavailability of zinc from Philippine rice. In a cooperative

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National Program(s): 107 100%

study, the zinc bioavailability of rice provided from the Philippine Rice Research Institute was determined in a rat study that also compared different bioavailability assessment methods. Relative to zinc sulfate (set at 100% bioavailability), zinc from well-milled (9-10% extraction, 0.165 mg Zn/g), under-milled (5-6% extraction. 0.194 mg Zn/g) and brown rice (0% extraction, 0.272 mg Zn/g) was 90, 83, and 74% as bioavailable to support rat growth; 91, 90, and 72% as available to support bone zinc incorporation; and 98, 89, and 87% as available to support whole body zinc retention, respectively. These findings support the use of brown rice, which would contribute more, although somewhat less bioavailable zinc than the refined forms of rice.

5. Describe the major accomplishments over the life of the project, including their predicted or actual impact.

In an iron supplementation trial we demonstrated that healthy people absorb much less nonheme iron, but the same amount of heme iron, from food when they are taking a daily iron supplement. Even women with low iron stores absorbed less nonheme iron from food when they took a daily iron supplement and the small increase in their iron stores with the iron supplementation lasted only 3 months after supplementation was stopped. This indicates that young women with low iron stores may need long-term iron supplementation or more available sources of iron like meat to counterbalance iron lost through menstruation. In a well-controlled feeding study, we demonstrated that healthy men also adapted to absorb less iron from a diet that provided highly available iron and absorbed more iron from a diet that provided poorly available iron. These findings of the ability of the intestine to adapt iron absorption up or down depending on one's diet are helpful in setting recommended dietary allowances for iron.

Determined that women of child-bearing age absorbed substantially less (about 70% less) nonheme iron from a vegetarian, compared with an omnivorous diet. Despite this large difference in iron absorption, the amount of iron in the body, as measured by serum ferritin, did not change. The findings from this study indicated that eating a vegetarian diet for 2 months does not decrease a person's iron status, however, the effects of long term vegetarianism on iron status is not clear.

Demonstrated that the intestinal cells made more ferritin, an iron-containing protein which is lost in the feces, when people ate diets with available sources of iron and also when

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Mode Code: 5450-10-00 STP Codes: 5.1.3.2 60% 5.1.3.3 40%
National Program(s): 107 100%

they were supplemented with extra iron. This noninvasive gastrointestinal marker which is analyzed in the stools can help us understand how iron absorption is controlled and which factors alter this control; this knowledge will be useful in the determination of intakes required for achievement of a healthy iron status.

Demonstrated that diets high in iron, within the amounts used to enrich, fortify and supplement human diets had minimal or no effect on risk factors for chronic disease in rats. This finding is important because high iron diets are thought to be a risk for the development of heart disease, cancer and other chronic diseases in humans.

6. What do you expect to accomplish, year by year, over the next 3 years?

Year 1: We will determine if women of childbearing age adapt to variations in dietary iron bioavailability, and whether adaptation is limited to the specific dietary enhancers and inhibitors of iron absorption, or constitutes a more general change in absorptive efficiency. Detailed dietary assessment data on 300 women will determine whether dietary variables (such as meat) that affect short-term dietary bioavailability can be related to long-term iron stores. We will test the hypothesis that, unlike purified sources of protein, meat consumption does not reduce human body calcium retention.

Year 2: We will test a new experimental approach to determining zinc requirements in humans, by determining how people adapt their zinc absorption to different dietary zinc intakes. We will determine whether people with the common genetic mutation associated with the iron storage disorder, hemochromatosis, absorb more iron than those without the mutation.

Year 3: We will begin measuring iron excretion rates for women, based on isotopic dilution over a 3-year period, and will relate iron excretion to body iron stores. We will test the usefulness of different chemical forms of iron supplements, especially heme iron, in increasing body iron stores without producing gastrointestinal oxidative damage.

7. What science and/or technologies have been transferred and to whom? When is the science and/or technology likely to become available to the end user (industry farmer, other scientists)? What are the constraints, if known, to the adoption and durability of the technology products

Research results were transferred to several customer groups, including food producers, other scientists, and health professionals and educators in nutrition, dietetics, and

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Accession: 0400398 Year: 00 Project Number: 5450-51000-021-00 D
Mode Code: 5450-10-00 STP Codes: 5.1.3.2 60% 5.1.3.3 40%
National Program(s): 107 100%

pharmacology through publications and presentations as listed in question 8 below.
ZK Roughead discussed "Osteoporosis, calcium, and meat" as a guest on the Terry Dullum Show, WDAZ, November 8, 1999.
ZK Roughead wrote a newspaper article: "Is eating meat good or bad for our bones?," GF Herald, November, 1999.

8. List your most important publicatons in the popular press (no abstracts) and presentations to nonscientific organizations and articles written about your work (NOTE: this does not replace your peer-reviewed publications which are listed below)

Research on iron absorption from a vegetarian diet (AJCN 69: 831,1999) was featured in The Arbor Nutrition Guide, an on-line literature update for nutrition professionals, on September 5, 1999.
Research on the effect of iron supplementation on iron absorption from food was featured in "idInsight," a newsletter of the Iron Disorders Institute, 4th quarter 1999, in an article "Absorption: Too much for some; too little for others."
JR Hunt presented "Supplements and Older Adults" and "Mineral Needs in Aging." Meeting of the Minnesota Northwest District Dietetic Association, Halstad, MN, February 4, 2000.

9. Scientific Publications:

01. Hunt, J.R. Roughead, Z.K. Adaptation of iron absorption in men consuming diets with high or low iron bioavailability. American Journal of Clinical Nutrition. 2000. v. 71. p. 94-102.

02. Roughead, Z.K., Zito, C.A., Hunt, J.R. Human absorption of nonheme iron is primarily determined by initial mucosal uptake, not mucosal transfer. FASEB Journal. 2000. v. 14. p. A297.

03. Hunt, J.R., Vanderpool, R.A. Copper absorption from a lactoovovegetarian diet. 2000. FASEB Journal. 2000. v. 14. p. A297.

04. JR Hunt presented "Bioavailability of Nutrients in Foods" USDA-ARS National Human Nutrition Research Program Planning Workshop, Washington, D.C., March 7-9, 2000.

05. JR Hunt presented "Dietary Supplement Use in Women: Responsible Strategies for Use" American Pharmaceutical Association Annual Meeting and Exposition, Washington, D.C., March 11, 2000.

Approved: FORREST H NIELSEN Date: 09/00
Title: CENTER DIRECTOR

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0401621 Year: 00 Project Number: 5450-51000-021-01 S
Mode Code: 5450-10-00 STP Codes: 5.1.3.2 60% 5.1.3.3 40%
National Program(s): 107 100%

Title: WHOLE BODY COUNTING AND RADIOTRACER METHODS IN RESEARCH ON MINERAL REQUIREMENTS IN HUMAN NUTRITION

Period Covered From: 10/99 To: 09/00

Would you like to terminate this Project? N

Will this project terminate within the first 2 months of FY2001? N

Progress and Outcomes:

1. What major problem or issue is being resolved and how are you resolving it?

Dietary recommendations and evaluation of dietary practices that promote good mineral nutrition for the population are made difficult by insufficient sensitive methods for measuring mineral nutrient absorption, excretion, retention, and food bioavailability. Whole body scintillation counter methods will be developed and used to safely and sensitively determine whole body retention of mineral elements that have gamma-emitting isotopes with short to moderate half-lives, such as cadmium, calcium, copper, iron, magnesium, manganese, and zinc. The whole body counting approach will determine mineral retention without volunteer inconvenience, high variability, and incomplete sample collections associated with collecting mineral excretion data. This agreement provides the expertise of a certified health physicist to cooperate with nutrition scientists at the Grand Forks Human Nutrition Research Center, providing an interdisciplinary approach to answering nutrition questions with specific and sensitive whole body counting methods.

2. How serious is the problem? Why does it matter?

Nutrient bioavailability addresses our ability to effectively utilize the nutrients in food for body biological functions. Two diets that contain similar amounts of a nutrient, such as iron, can differ by as much as 10-fold in the amount of iron that is actually absorbed, retained, and utilized by the body. Internationally, zinc deficiency has been observed in humans whose diets contained considerable quantities of zinc, but that zinc was not bioavailable because of phytic acid from whole grains or legumes that interfered with absorption, and the lack of protein that enhanced absorption. Domestically, there is concern that dietary trends (and some recommendations) to increase whole grains and legumes while reducing animal products in the diet may

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National Program(s): 107 100%

compromise copper, iron and zinc nutrition. The promotion of mineral supplements in the US may lead to imbalances that affect the absorption and retention of other minerals. To complement new knowledge of the importance of nutrients for optimal health, we must also know the bioavailability of and interactions among nutrients from common diets, which whole body counting techniques will allow us to determine, in order to provide dietary advice to the public.

3. How does it relate to the National Program(s) and National Program Component(s)?

This agreement fits in the National Program 107, Human Nutrition (100%) Research conducted is directly related to ARS National Human Nutrition Performance Goals 3.1.1, Human Nutrition Requirements, and 3.1.3, Nutritious Plant and Animal Products, and emphasizes the primary objective: Bioavailability of Nutrients and Food Components.

4. What were the most significant accomplishments this past year?

We evaluated whether the level of background radiation in the whole body counter could be reduced, thereby further optimizing the accuracy of absorption and retention measurements made by the whole body counter. The natural gamma ray background radiation at the GFHNRC was quantitatively compared with that at Lawrence Livermore National Laboratory (LLNL), Livermore, California, by recording measurements using two NaI(Tl) detectors at the two locations. It was determined by using a sealed, "Golden Standard" reference detector, as well as by a high-purity germanium detector, that the gamma ray background radiation in the steel room of the whole body counter was adversely affected by eliminating forced air flow (probably as a result of accumulating radiation from the lead lining of the room), as well as by doubling forced air flow (probably as a result of increased radiation from air-born radon and its decay products). These results confirm that the air flow of the room is set for optimal performance to support isotopic tracer studies of mineral nutrition in humans, which this year involved studies of calcium, iron and zinc absorption and retention.

5. Describe the major accomplishments over the life of the project, including their predicted or actual impact.

The quality of data collection by the whole body counter was

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National Program(s): 107 100%

improved by using a new Uniform Isotope Source (UNIS) board, and by integration of a new algorithm employing a "three-gamma ray" UNIS board into Library Least Squares programs. This method eliminates variability introduced by subject size and radioisotope distribution which can be a source of error in whole body counting methodology.

DOS-based computer programs were developed for use with the repaired small animal whole body counter, and recently the software was upgraded to a Windows-based, user-friendly program which will incorporate standard "off the shelf" sample calibration algorithms.

An in-depth quality control study of the GFHNRC whole body counting equipment was conducted through a student participating in the UND 1998 Research Experience for Undergraduates in Physics (REU) Program. Time-based trends showed, as expected, decreased sensitivity and performance of the whole body counter detectors.

A large 12.5 cm x 5 cm NaI(Tl) cylindrical detector was obtained and methods were developed to selectively measure cranium and other organ gamma ray emissions in metabolic studies employing radioactive tracers. This equipment, including two shielded counting chambers, a high purity germanium (HpGe) and sodium-iodide [NaI(Tl)]detectors and associated electronics, afford independent analytical identification and standardization of isotope sources. The continuous improvement in whole body counting methods provides a unique resource for extending knowledge of mineral absorption and retention.

Precise whole body counting measurements allowed sensitive measurements of iron retention, revealing that men adapt iron absorption in response to changes in dietary iron bioavailability. These results demonstrated that research with short-term diets overestimates differences in iron bioavailability found with long-term diets. Additional studies with the whole body counter demonstrated that people supplemented with iron adapt to reduce the amount of iron they absorb from food, and that people consuming low manganese diets adapt to absorb manganese more efficiently. These findings can be useful in formulating recommended dietary allowances for iron and manganese. See CRIS 5450-51000-020-00D and 5450-51000-021-00D.

6. What do you expect to accomplish, year by year, over the next 3 years?

Year 1: The library least squares method will be further developed to establish minimal detection limits for specific radioisotopes measured against a typical K-40 background, and

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National Program(s): 107 100%

to utilize the new algorithm based on the "three-gamma ray" UNIS board. This work will improve the precision of the whole body counter and aid data analysis and interpretation of the variation caused by body size and density in self-absorption of internally produced radiation. These improvements will make whole body counting more useful in determining changes in body composition associated with diet and/or exercise over time.

Year 2: The retention of cadmium, consumed in amounts that occur naturally in US crops such as wheat and sunflower seeds will be determined in humans.

Year 3: The whole body distribution of calcium-47 (including its retention and different routes of excretion)will be determined by using whole body counting and the newly acquired high purity germanium detector.

7. What science and/or technologies have been transferred and to whom? When is the science and/or technology likely to become available to the end user (industry farmer, other scientists)? What are the constraints, if known, to the adoption and durability of the technology products

Because of the uniqueness of the whole body counting equipment and technology, the major transfer of technologies from the whole body counter work have occurred through reports and publications of the results of the major nutrition projects which employed the counter, and are reported under those specific CRIS reports. Application information was shared through discussions with scientists at International Symposia, physicists in academia as well as Medical Physicists at Brookhaven National Laboratory. A perquisite spin-off from studies of the steel room background radiation has led to research into contamination of lead used in contacts on high speed, high density semiconductor computer memory elements. Radiation from contaminated lead contacts leads to errors in the memory elements ("soft errors"). This work is supported by The Ballistic Missile Defense Organization through the Department of Defense Experimental Program to Stimulate Competitive Research (DEPSCoR). Clean lead contacts, "solder bumps" in 'flip chip' memory elements will result in faster, smaller, more accurate computer memories.

8. List your most important publicatons in the popular press (no abstracts) and presentations to nonscientific organizations and articles written about your work (NOTE: this does not replace your peer-reviewed publications which are listed below)

None

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Mode Code: 5450-10-00 STP Codes: 5.1.3.2 60% 5.1.3.3 40%
National Program(s): 107 100%

9. Scientific Publications:

01. Momcilovic, B., Alkhatib, H.A., Duerre, J.A., Cooley, M.A., Long, W.M., Harris, R.T., Lykken, G.I. Environmental radon daughters reveal pathognomonic changes in the brain proteins and lipids in patients with Alzheimer's Disease and Parkinson's Disease, and Cigarette smokers. Archives of Occupational Health and Toxicology. 1999. v. 50. pp. 347-369.

02. Lykken, G.I., Ong, H.S., Alkhatib, H.A., Harris, R.T., Momcilovic, B, Penland, J.G. Perquisite spin-off from twenty-two years of measuring background in the whole body counter steel room. In vivo body composition studies. Annals of the New York Academy of Sciences. 2000. v. 904. pp. 267-271.

03. Lykken, G.I. "What 'soft' errors in computer memories and 'hard' errors in human memories may have in common with environmental radon" presented at the Physics Department, University of Winnipeg, Winnipeg, Canada, January 10, 2000.

04. Lykken, G.I. "What 'soft' errors in computer memories and 'hard' errors in human memories may have in common with environmental radon" presented at the Grand Forks Human Nutrition Research Center on February 9, 2000.

05. Hatfield, A.L. and Lykken, G.I."A study of factors affecting whole body counter steel room background" presented at the North Dakota Science, Engineering and Mathematics 7th Annual Poster Session Program, North Dakota State University, Fargo, ND, July 28, 1999.

06. Lykken, G.I. "From "Clean" Galena to "Contaminated" Lead-Why?" and "Micron Slices of Lead for Assessment of Alpha Particle Emissions in Computer Chip Manufacturing" presented the Minerals-Materials-Metals, TMS, Society Annual Meeting, Nashville, TN, March 12-16, 2000.

Approved: FORREST H NIELSEN Date: 09/00
Title: CENTER DIRECTOR

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ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0401160 Year: 00 Project Number: 5450-51000-021-03 T
Mode Code: 5450-10-00 STP Codes: 5.1.3.2 60% 5.1.3.3 40%
National Program(s): 107 100%

Title: ADAPTATION IN THE ABSORPTION OF IRON FROM BEEF

Period Covered From: 10/99 To: 09/00

Would you like to terminate this Project? N

Will this project terminate within the first 2 months of FY2001? N

Progress and Outcomes:

1. What major problem or issue is being resolved and how are you resolving it?

Concerns about high iron stores increasing the risk of chronic diseases such as heart disease and cancer have led to the speculation that red meat intake should be limited because it is an excellent source of highly absorbable iron in the heme form. There is special concern that heme iron absorption is not biologically controlled to the same degree as nonheme iron absorption. This project is related to parent CRIS 5450-51000-021-00D by the same investigators, and is limited to a single human experiment that has been partially funded by the National Cattlemen's Beef Association. The objective of this experiment is to determine a) whether heme iron absorption from a meat-based meal is reduced after iron supplementation, b) whether nonheme iron absorption from a meat-based meal is reduced after iron supplementation, c) whether intestinal ferritin production, as measured in fecal samples, is increased after iron supplementation, and is associated with changes in heme and nonheme iron absorption, and d) whether serum ferritin is increased substantially after iron supplementation, and if any increase in serum ferritin persists after iron supplementation is discontinued.

2. How serious is the problem? Why does it matter?

Iron nutrition is a double-edged sword. While adequate iron nutriture in infants, children, and childbearing age women is essential for cognitive development and work capacity, excessive body iron in men and postmenopausal women is hypothesized to increase oxidative stress and the risk of chronic diseases such as heart disease and cancer. Research on dietary iron bioavailability provides information for policy decisions about recommended dietary allowances, nutrient supplementation, food enrichment and fortification standards, and dietary guidelines for the public. Results from this research may suggest that red meat is a good source of iron

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Accession: 0401160 Year: 00 Project Number: 5450-51000-021-03 T
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National Program(s): 107 100%

for persons with inadequate iron nutriture, without providing excessive iron for those with adequate iron stores.

3. How does it relate to the National Program(s) and National Program Component(s)?

The research fits into the National Program 107, Human Nutrition (100%), is directly related to ARS National Human Nutrition Performance Goals 3.1.1, Human Nutrition Requirements, and 3.1.3, Nutritious Plant and Animal Products, concerning the primary objective: Bioavailability of Nutrients and Food Components.

4. What were the most significant accomplishments this past year?

None

5. Describe the major accomplishments over the life of the project, including their predicted or actual impact.

We found that healthy people, even those with low iron stores, reduced the total amount of iron they absorbed from a meat-based meal in response to iron supplementation. The reduction was in nonheme, but not heme iron absorption. Fecal ferritin, an iron-containing protein made by the intestinal cells (which is lost in the stools when the cell dies) increased with iron supplementation. Individuals with adequate iron stores did not fully adapt to prevent increased iron stores with supplementation, and those with low iron stores rapidly reverted to their previous iron status after supplementation was discontinued. We also found that the participants with low iron stores (all women)absorbed more heme iron than those with higher iron stores both at the beginning and at the end of the supplementation. Therefore, the notion that heme iron consumption would lead to iron overload in healthy people was not supported. We also concluded that heme iron may be an important source of bioavailable iron for women with low iron stores. This research contributes to a better understanding of the control of body iron absorption and emphasizes the need for dietary guidelines as well as fortification or supplementation practices that consider adaptation processes and bioavailability in making recommendations to protect against iron deficiency and iron excess.

6. What do you expect to accomplish, year by year, over the next 3 years?

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Accession: 0401160 Year: 00 Project Number: 5450-51000-021-03 T
Mode Code: 5450-10-00 STP Codes: 5.1.3.2 60% 5.1.3.3 40%
National Program(s): 107 100%

(Not applicable. This project will be completed with the publication of a technical scientific paper in the last quarter of 2000.)

7. What science and/or technologies have been transferred and to whom? When is the science and/or technology likely to become available to the end user (industry farmer, other scientists)? What are the constraints, if known, to the adoption and durability of the technology products

The new finding that people partially adapt to iron supplementation by reducing their iron absorption from food was transferred to several customer groups, including food producers, other scientists, and health professionals in nutrition, dietetics, and pharmacology through presentations as listed in question 8 below.
JR Hunt wrote an article about nutritional balance and adaptation in iron absorption in "You can't fool Mother Nature," Grand Forks Herald, November 24, 1999.
ZK Roughead presented: "New information about an old nutrient: iron." Department of Anatomy, University of North Dakota, Grand Forks, ND, May 2000.
JR Hunt presented "Iron, A Double-Edged Sword" Minnesota Dietetic Association Annual Meeting, Rochester, MN, May 5, 2000.
JR Hunt presented "Research Needs Relative to Dietary Zinc and Iron and the Role of Dietary Beef," at a symposium sponsored by the National Cattlemen's Beef Association, Chicago, IL, September 27, 2000.

8. List your most important publicatons in the popular press (no abstracts) and presentations to nonscientific organizations and articles written about your work (NOTE: this does not replace your peer-reviewed publications which are listed below)

Research on the effect of iron supplementation on iron absorption from food was featured in "idInsight", a newsletter of the Iron Disorders Institute, 4th quarter 1999, in an article "Absorption: Too much for some; too little for others."

9. Scientific Publications:

Approved: FORREST H NIELSEN Date: 09/00
Title: CENTER DIRECTOR

UNOFFICIAL

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0400524 Year: 00 Project Number: 5450-51000-022-00 D
Mode Code: 5450-10-00 STP Codes: 5.1.3.3 50% 5.1.3.4 50%
National Program(s): 107 100%

Title: HUMAN MINERAL ELEMENT REQUIREMENTS AND THEIR MODIFICATION BY STRESSORS

Period Covered From: 10/99 To: 09/00

Would you like to terminate this Project? N

Will this project terminate within the first 2 months of FY2001? N

Progress and Outcomes:

1. What major problem or issue is being resolved and how are you resolving it?

The extent to which dietary mineral excesses, deficiencies and imbalances contribute to the susceptibility and severity of a number of chronic diseases of major health and economic consequence has not been established; the chronic diseases include coronary heart disease, hypertension, stroke, atherosclerosis, and osteoporosis. Additionally, the extent to which mineral element nutrition contributes to these diseases is unknown when nutritional, metabolic, hormonal or physiological stressors are present which could enhance the need, or interfere with the utilization of the mineral elements. There is a need to ascertain the validity of claims that magnesium is of practical concern for maintaining bone and cardiovascular health, especially when diets are low in copper and/or high in fructose; that boron, copper, zinc and manganese status affects calcium utilization and metabolism and thus the susceptibility of osteoporosis; and that high dietary zinc adversely affects copper metabolism resulting in an increased risk to cardiovascular disease.

Studies with human volunteers are being and will be conducted. These studies include the examining of the effects of varying intakes of zinc at different intakes of dietary copper on lipid profiles, bone status indicators and reactive oxygen metabolism; of whether low magnesium intakes with and without stressors results in a neurogenic inflammatory response leading to oxidative damage that can lead to pathophysiology such as cardiomyopathy, migraine headaches, and abnormal central nervous system function; and whether boron supplementation of individuals with suspected low boron status improves cognitive and motor function and indicators of bone health.

2. How serious is the problem? Why does it matter?

Dietary factors, including trace element nutriture, are

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National Program(s): 107 100%

associated with 5 of the 10 leading causes of death. Among the diseases that are closely linked to diet, the cost for treatment and care in the United States exceeds $200 billion per year. Among the diseases associated with subnormal mineral element nutrition, the annual economic cost is estimated to be greater than $80 billion for cardiovascular disease, and $10 billion for osteoporosis. Several mineral elements associated with these chronic diseases including boron, copper, magnesium and zinc have been shown to be routinely low in the diets in the United States. Thus, providing information about requirements and factors that affect those requirements of critical mineral elements should result in policies and programs that improve intakes of these nutrients that will result in a healthier population, decrease the burden of chronic disease, enhance the quality of life, and diminish health care expenditures.

3. How does it relate to the National Program(s) and National Program Component(s)?

The research relates to the National Program 107, Human Nutrition, and specifically to the program component of Nutrient Requirements. The research helps determine mineral requirements that prevent disease and promote health and optimal function throughout life. The major focus is on determining the biochemical and health consequences of suboptimal mineral intakes, with the objective of showing that mineral nutrition influences the major chronic, degenerative conditions associated with aging.

4. What were the most significant accomplishments this past year?

A. Single most significant accomplishment during FY 2000.
Although low dietary calcium receives the most recognition for increasing the risk of developing osteoporosis, there are a number of other mineral elements, including copper and zinc, whose lack could increase the susceptibility to this disorder. Bone status and calcium utilization indicators were determined in healthy postmenopausal women that were fed either 1 or 3 mg Cu/day and 3 mg Zn/day for 90 days, then 53 mg of Zn for the next 90 days. Low zinc, low copper, and especially the combined low zinc and copper caused unfavorable changes in bone and calcium status indicators including urinary N-telopepetides and calcium, and plasma osteocalcin and ionized calcium. The findings indicate that increasing the dietary intake of copper and zinc is an action a significant number of people could do to reduce the

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National Program(s): 107 100%

risk of developing osteoporosis.
B. Other significant accomplishments
None.

5. Describe the major accomplishments over the life of the project, including their predicted or actual impact.

Consumption of a diet rich in polyunsaturated fat (vegetable fats tend to be more polyunsaturated) significantly increased plasma ionized calcium, ionized magnesium and total magnesium concentrations and significantly decreased plasma iron concentration and transferrin saturation in postmenopausal women. IMPACT: The findings show that changes in dietary fat can modify the metabolism of minerals associated with bone maintenance and growth and with iron utilization. Routine consumption of a diet high in polyunsaturated fats may help reduce the risk of osteoporosis, but also may increase the risk of becoming iron deficient if iron intake is low.

Experimental evidence using human volunteers shows that low dietary intakes of magnesium induces changes in indices used to assess the susceptibility to cardiovascular and calcium metabolism disorders; changes induced include heart rhythm abnormalities, altered cardiovascular function and energy metabolism in postmenopausal women. IMPACT: Because magnesium is often consumed in inadequate amounts according to dietary surveys, these findings indicate that magnesium is of practical nutritional and clinical importance in the maintenance of healthy cardiovascular system, and thus in the prevention of heart disease.

Calcium balance can be maintained in postmenopausal women with intakes less than 800 mg/day, but can be undesirably altered by low intakes of copper, zinc and magnesium. IMPACT: These findings show that high dietary intakes of calcium are not the complete answer to the prevention of bone loss leading to osteoporosis. If nutrients such as magnesium, copper, zinc and boron are consumed in appropriate amounts, high dietary calcium intakes (such as those indicated by the new Dietary Reference Intakes) difficult to achieve by diet alone are unnecessary to prevent bone loss in postmenopausal women.

The consumption of high amounts of fructose decreases calcium balance with the effect more marked when dietary magnesium is low. IMPACT: This finding indicates that the consumption of high amounts of carbonated beverages sweetened with high fructose corn syrup is detrimental to the formation and maintenance of healthy strong bones.

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Accession: 0400524 Year: 00 Project Number: 5450-51000-022-00 D
Mode Code: 5450-10-00 STP Codes: 5.1.3.3 50% 5.1.3.4 50%
National Program(s): 107 100%

High dietary zinc (53 mg/day) compared to low dietary zinc (3 mg/day) significantly decreased plasma cholesterol and significantly increased a number of indicators of copper status including platelet cytochrome c oxidase activity and ceruloplasmin concentrations in postmenopausal women fed low (1 mg/day) or luxuriant (3 mg/day) copper. IMPACT: These findings do not support the dogma that a moderately high dietary zinc intake adversely affects copper metabolism, but show that a low dietary zinc intake does. Combined low intakes of copper and zinc may be important factors in the occurrence of heart disease.

6. What do you expect to accomplish, year by year, over the next 3 years?

In FY 2001, a human study will be completed which will ascertain whether magnesium deprivation results in neurogenic dysfunction that induces a number of other undesirable changes including enhanced susceptibility to oxidative stress and detrimental inflammatory responses, which could lead to heart rhythm changes, sleep disorders, mood disturbances and osteoporosis.

In FY 2002, a human study will be completed which will confirm that low dietary zinc is an important factor in inducing changes in copper status indicators, and that a combined copper and zinc deprivation results in changes that could lead to increased susceptibility to osteoporosis and heart disease.

In FY 2003, human studies will be initiated to determine whether the mineral elements nickel and silicon are of nutritional importance.

7. What science and/or technologies have been transferred and to whom? When is the science and/or technology likely to become available to the end user (industry farmer, other scientists)? What are the constraints, if known, to the adoption and durability of the technology products

Information about the nutritional and clinical importance of the mineral elements magnesium, copper, boron and zinc as it becomes available is routinely transferred to a variety of customers. The customers include the public through web pages of professional organizations and via the popular media, and other scientists through presentations at national and international meetings and professional publications.

The presentation "Nutritional Aspects of Osteoporosis" was given to the Grand Forks Human Nutrition Research Staff, March 15, 2000.

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Accession: 0400524 Year: 00 Project Number: 5450-51000-022-00 D
Mode Code: 5450-10-00 STP Codes: 5.1.3.3 50% 5.1.3.4 50%
National Program(s): 107 100%

8. List your most important publicatons in the popular press (no abstracts) and presentations to nonscientific organizations and articles written about your work (NOTE: this does not replace your peer-reviewed publications which are listed below)

The presentation "Balderdash and Realities of Nutritional Supplements" given to the Franklin Club, Grand Forks, ND, October, 1999.
Research findings cited in periodicals including Bottom Line Personal, Remedy, and the Grand Forks Herald.

9. Scientific Publications:

01. Nielsen, F.H. The importance of making dietary recommendations for elements designated as nutritionally beneficial, pharmacologically beneficial, or conditionally essential. Journal of trace elements in experimental medicine. 2000. v. 13. p. 113-129.

02. Nielsen, F.H., Davis, C.D. and Milne, D.B. Low dietary zinc and copper negatively affect plasma and urine indicators of bone health. Proceedings of the North Dakota Academy of Science. 2000. v. 54. p. 39.

03. Nielsen, F.H. Evolutionary events culminating in specific minerals becoming essential for life. European journal of nutrition. 2000. v. 39. p. 62-66.

04. Nielsen, F.H. The emergence of boron as nutritionally important throughout the life cycle. Nutrition. 2000. v. 16. p. 512-514.

05. Nielsen, F.H. Possibly essential trace elements. IN: J.D. Bogden and L.M. Klevay (eds.) Clinical Nutrition of the Essential Trace Elements and Minerals - The Guide for Health Professionals, Humana Press, Totowa, NJ. 2000. p. 11-36.

06. Nielsen, F.H. The dogged path to acceptance of boron as a nutritionally important mineral element. IN: 10th International Symposium on Trace Elements in Man and Animals - TEMA 10, Book of Abstracts, Evian, France. Abstr. p. 94.

07. Nielsen, F.H. presented "Low dietary zinc and copper negatively affect plasma and urine indicators of bone health" at the 92nd Annual Meeting of the North Dakota Academy of Science, Moorhead, MN, April 29, 2000. Moorhead, MN. April 29, 2000.

Approved: FORREST H NIELSEN Date: 09/00
Title: CENTER DIRECTOR

UNOFFICIAL

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0403096 Year: 00 Project Number: 5450-51000-024-00 D
Mode Code: 5450-10-00 STP Codes: 5.1.3.4 100% %
National Program(s): 107 100%

Title: RELATIONSHIP BETWEEN MINERAL NUTRITION AND RISK FACTORS FOR CANCER

Period Covered From: 10/99 To: 09/00

Would you like to terminate this Project? N

Will this project terminate within the first 2 months of FY2001? N

Progress and Outcomes:

1. What major problem or issue is being resolved and how are you resolving it?

Diet affects cancer risk; however, it is unknown how suboptimal mineral element nutrition may contribute to the susceptibility of cancer. Thus, a better understanding of the mechanism and the optimal amounts of trace minerals for cancer prevention could help decrease the burden of cancer in the United States. One possible dietary factor that may increase the susceptibility to colon cancer is inadequate dietary copper. Recent studies have shown that ingestion of a diet deficient in copper significantly increased the risk of colon cancer in experimental animals treated with chemical carcinogens. This indicates low dietary copper may be a potential risk factor for colon cancer in humans. In addition, epidemiologic studies have shown that selenium supplementation decreased the risk of and mortality from cancer at a number of sites. A better understanding of the mechanism for the protective effect of selenium, the form of selenium which is most efficacious, and the optimal amount of selenium for cancer prevention is needed.

Min (multiple intestinal neoplasia) mice resemble a hereditary form of human colon cancer. These mice contain a mutation in the murine homolog of the human APC gene and develop spontaneous tumors throughout the intestine. This genetic model for cancer susceptibility will be used to study the effects of trace minerals including copper and selenium on the pathogenesis of intestinal cancer. The effects of changes in mineral status on susceptibility of humans to colon cancer will be investigated by feeding people different diets and analyzing the fecal water fraction for cytotoxicity, genotoxicity and alkaline phosphatase activity.

2. How serious is the problem? Why does it matter?

Cancer is the second leading cause of death in the United States. It has been estimated that the cost for the treatment

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0403096 Year: 00 Project Number: 5450-51000-024-00 D
Mode Code: 5450-10-00 STP Codes: 5.1.3.4 100% %
National Program(s): 107 100%

and care of this disease exceeds $100 billion per year. In addition to the economic impact, the development of cancer may prevent many from enjoying life to its fullest. It is believed that diet is the single greatest contributor to human cancer, possibly accounting for 35-45% of the disease. Dietary excesses, deficiencies and imbalances in trace mineral intake are factors that can affect cancer susceptibility. Thus, providing information about requirements and factors that affect the formulation of dietary guidance for mineral elements should result in policies and programs that improve intakes of these nutrients that will result in a healthier population, decrease the burden of chronic disease, enhance the quality of life, and diminish health care expenditures.

3. How does it relate to the National Program(s) and National Program Component(s)?

This research is related to the National Program 107, Human Nutrition Requirements (100%) and addresses the specific National Program Component of the Human Nutrition Action Plan, "Diet, Genetics and Lifestyle and the Prevention of Obesity and Disease." One of the primary objectives of this component is to "identify the nutrient-relevant genetic, epigenetic and environmental nutrients on gene expression and developmental programming that have permanent consequences on human health and disease."

4. What were the most significant accomplishments this past year?

A. Single Most Significant Accomplishment during FY 2000 year:
Low dietary copper has been shown to increase the risk of colon cancer development in experimental animals and to decrease the expression of various protein kinase C isozymes, a series of proteins involved in the signal transduction pathway within the cell. An animal study investigated the relationship between dietary copper and carcinogen administration on protein kinase C expression and the appearance of preneoplastic lesions for colon cancer. Low dietary copper increased the formation of preneoplastic lesions for colon cancer and decreased protein kinase C expression. The current finding provides a biochemical explanation for the increased incidence of precancerous lesions in colons of copper-deficient rats when they are challenged with a carcinogen, and suggests that low dietary copper may be a risk factor for colon cancer susceptibility in humans because more than 60% of the diets in the U.S. fail to provide the recommended amount of copper.

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National Program(s): 107 100%

B. Other Significant Accomplishment(s), if any:

Familial adenomatous polyposis (FAP) is a human disease that has been linked to changes in the APC gene known as mutations; individuals possessing these mutations develop numerous intestinal polyps (precancerous lesions) at an early age. Min mice (multiple intestinal neoplasia mice) carry a mutation in what is equivalent to the human APC gene and develop intestinal tumors similar to those found in patients with familial adenomatous polyposis syndrome and are therefore a good model for the investigation of the effects of dietary alterations on genetic susceptibility for intestinal cancer. Min mice that were fed a copper deficient diet had significantly more and bigger tumors compared to similar mice fed adequate copper. These results reveal that adequate dietary copper can protect animals against a genetic form of cancer; adequate dietary copper may help people with a similar genetic illness.

Decreases in the amount of methylation of DNA are associated with increased cancer susceptibility. Studies were conducted to determine whether changes in the amount and chemical form of selenium would affect the methylation of DNA in experimental animals and/or in a human colon cancer cell line and whether this effect would be modified by arsenic. DNA isolated from animals and cells exposed to deficient selenium had significantly lower DNA methylation than DNA isolated from animals and cells exposed to adequate selenium. These results suggest that alterations in DNA methylation may be a potential mechanism whereby deficient dietary selenium increases cancer susceptibility.

5. Describe the major accomplishments over the life of the project, including their predicted or actual impact.

The frequency of carcinogen-induced preneoplastic (precancerous) lesions (aberrant crypt foci) associated with colon cancer development was significantly increased in animals fed low dietary copper and tended to be increased in animals fed low dietary manganese and high dietary iron. Altered activities of antioxidant enzymes, known as superoxide dismutases, were significantly correlated with the number of the anatomical lesions associated with colon cancer. These findings suggest that dietary alterations which affect superoxide dismutase activity affect cancer susceptibility. Furthermore, the effect of dietary copper and manganese on aberrant crypt foci formation may have practical implications because diets in the United States often contain copper and

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National Program(s): 107 100%

manganese in amounts less than their estimated safe and adequate daily dietary intakes.

Demonstrated that the amount and the chemical form of selenium in the diet can influence colon cancer susceptibility. Supplementation of a selenium deficient diet with either selenite or selenate but not selenomethionine caused a significant reduction in the amount of carcinogen-DNA adducts in the colon but not in the liver of rats. The formation of carcinogen-DNA adducts is necessary for the first step in cancer development. The reduction in carcinogen-DNA adduct formation in the colon correlated with a reduction in aberrant crypt foci when animals were fed selenite or selenate but not selenomethionine

6. What do you expect to accomplish, year by year, over the next 3 years?

In FY 2001, a human study will be conducted to determine the effect of low dietary copper on susceptibility of humans to colon cancer. Subjects will collect their feces and the fecal water fraction will be analyzed for cytotoxicity, genotoxicity and alkaline phosphatase activity. These assays will be used as indicators of cancer susceptiblity in the whole individual. Animal studies will be used to determine the mechanism for the protective effects of dietary selenium and copper against colon cancer susceptibility. Animals will be fed different amounts and chemical forms of selenium and their colons will be analyzed for cancer susceptibility, DNA methylation and selenium containing enzyme activity.

In FY 2002, the analysis of all samples obtained from the human study performed in FY 2001 will be completed. In vitro experiments will be conducted to determine whether changes in media trace minerals will affect the proliferation, differentiation, DNA methylation and tumorigenicity of human colon cancer cells.

In FY 2003, a human supplementation study will be conducted to assess the protective effects of different food forms of selenium on colon cancer susceptibility.

7. What science and/or technologies have been transferred and to whom? When is the science and/or technology likely to become available to the end user (industry farmer, other scientists)? What are the constraints, if known, to the adoption and durability of the technology products

Information about the effect of trace minerals on cancer susceptibility as it becomes available is routinely transferred to a variety of customers. The customers include the public through web pages of professional organizations and

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via the popular media, and other scientists through presentations at national meetings and professional publications.

Information was transferred to the public through an article, "Should you take supplements to decrease cancer risk?" published in the Grand Forks Herald, June, 2000 and placed on the web site of the Grand Forks Human Nutrition Research Center.

8. List your most important publicatons in the popular press (no abstracts) and presentations to nonscientific organizations and articles written about your work (NOTE: this does not replace your peer-reviewed publications which are listed below)

An article, "Colon cancer curbed by high-selenium broccoli" published in Agriculture Research, June, 2000.
An article, "High-selenium broccoli vs. colon cancer" published in Food and Nutrition Research Briefs, July 2000.

9. Scientific Publications:

01. Davis, C.D., Milne, D.B., Nielsen, F.H. Changes in dietary zinc and copper affect zinc-status indicators of postmenopausal women, notably, extracellular superoxide dismutase and amyloid precursor proteins. American Journal of Clinical Nutrition. 2000. v. 71. p. 781-8.

02. Davis, C.D., Johnson, W.T. 2000. Dietary copper and dimethylhydrazine (DMH) affect protein kinase C (PKC) isozyme expression in rat colon. Federation of American Societies for Experimental Biology Journal. 2000. v. 14. p. A169.

03. Davis, C.D. "Dietary copper and dimethylhydrazine (DMH) affect protein kinase C (PKC) isozyme expression in rat colon." Presented at the Experimental Biology 2000 Meeting, San Diego, CA, April 15-18, 2000.

Approved: FORREST H NIELSEN Date: 09/00
Title: CENTER DIRECTOR

UNOFFICIAL

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0149894 Year: 00 Project Number: 5450-51530-004-00 D
Mode Code: 5450-10-00 STP Codes: 5.1.3.3 25% 5.3.1.2 75%
National Program(s): 107 100%

Title: DEVELOPMENT AND EVALUATION OF METHODS FOR THE CLINICAL EVALUATION OF MINERAL NUTRITIONAL STATUS

Period Covered From: 10/99 To: 09/00

Would you like to terminate this Project? Y

Will this project terminate within the first 2 months of FY2001? N

Progress and Outcomes:

1. What major problem or issue is being resolved and how are you resolving it?

Although the essentiality of magnesium, copper and zinc is well established, the general consensus in the mineral research community is that there are currently no adequate clinical tests for evaluating the nutritional status of these elements in humans. Moreover, the lack of accurate diagnostic tests to assess magnesium, copper and zinc status has impeded the development of robust well-founded dietary guidance for these essential elements, and impeded the identification of chronic diseases, such as ischemic heart disease and osteoporosis, whose incidence and severity could involve imbalanced or deficient intakes of these essential mineral elements.

The approach to resolving the problem is to feed human volunteers deficient or imbalanced intakes of copper, zinc and/or magnesium, measure variables that can be ethically assessed and are responsive to deficient or imbalanced intakes based on findings from animal models, and compare the values obtained to those obtained when intakes of these elements are balanced and adequate.

2. How serious is the problem? Why does it matter?

Food consumption surveys indicate that a significant number of people in the United States have intakes of copper, zinc and/or magnesium that are below those recommended by knowledgeable groups such as the Food and Nutrition Board of the National Academy of Sciences. Yet, pathology caused by deficiencies of these elements is not readily recognized or diagnosed because the lack of definitive methods for identifying deficient states. Establishment of specific, accurate and cost-effective tests for the measurement of nutritional status will aid clinicians in detecting nutritional deficiencies and imbalances in their early stages. Early detection of inadequate trace element status would

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National Program(s): 107 100%

result in considerable savings in the cost for treatment and care of diet-related diseases in the United States that has been estimated to exceed $200 billion per year. Additionally, methods to assess status would be applicable to studies determining human requirements and metabolism of trace elements. Knowledge gained from this research project will facilitate the evaluation of federal food and nutrition programs, and the administration of programs that contribute to the health and well-being of people.

3. How does it relate to the National Program(s) and National Program Component(s)?

The research relates to the National Program 107, Human Nutrition, and specifically to the program component of Nutrient Requirements which states research is needed to identify biomarkers of nutrient intakes and status, nutritional adequacy and disease prediction. The research emphasizes priority objectives of Biomarkers, and Function and Performance, but also can apply to objectives of Mechanism of Action and Nutrient Interactions.

4. What were the most significant accomplishments this past year?

A study was conducted to evaluate possible indicators of low and sumptuous copper and zinc status. Status indicators were determined in healthy postmenopausal women that were fed either 1 or 3 mg copper/day and 3 mg zinc/day for 90 days, then 53 mg of zinc for the next 90 days. Immunoreactive ceruloplasmin concentrations in serum and platelet cytochrome c oxidase activity on a platelet number basis were significantly lower, and the ratio between enzymatic and immunoreactive ceruloplasmin was significantly higher during low dietary than during high dietary zinc intake. These changes in copper status indicators suggest that they might be useful in evaluating changes in zinc status in humans, and also indicate that an inadequate intake of zinc (3 mg/day) was more effective than a moderately high intake of zinc (53 mg/day) in inducing changes associated with a decreased copper status in postmenopausal women.

5. Describe the major accomplishments over the life of the project, including their predicted or actual impact.

Plasma zinc, extracellular superoxide dismutase, erythrocyte membrane 5'-nucleotidase, and bone specific alkaline phosphatase were shown to be sensitive indicators of zinc

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National Program(s): 107 100%

nutritional status in postmenopausal women fed 3 then 53 mg of zinc for 90 days each. These indicators should be helpful in defining human zinc requirements during different stages of the life cycle, and for surveys evaluating zinc status of populations at risk to chronic disease in which suboptimal zinc intake plays a role.

Magnesium status indicators were determined in a double blind crossover study in which postmenopausal women were fed low and deficient magnesium and low or deficient copper. The sequence of in which magnesium supplements were given obscured the magnesium effects on variables such as serum magnesium, serum ionized magnesium, serum cholesterol, plasma glucose, and red cell superoxide dismutase; changes were greatest when the placebo was fed first and were smaller or lacking when magnesium was fed first. The findings suggest that prior high magnesium intake and status inhibits or delays the appearance of magnesium deprivation signs. This information is useful in the development of further studies with the objective of determining magnesium nutritional requirements, and defining the consequences of magnesium deprivation.

High dietary fructose significantly increased magnesium balance and the urinary loss of phosphorus and depressed both calcium and phosphorus balances, with the effects more marked when dietary magnesium was low. These findings suggest that high dietary fructose adversely affects calcium and phosphorus metabolism in humans, particularly when dietary magnesium is low. This is of concern because recent surveys indicate that the consumption of fructose-based sweeteners is high and that a significant portion of the population is consuming diets low in magnesium. The findings suggest that high fructose intakes, such as that with a high intake of soda pop and sport drinks, coupled with a low dietary magnesium intake could result in the loss of bone, or osteoporosis.

6. What do you expect to accomplish, year by year, over the next 3 years?

This project needs to be terminated. The SY associated with the project has retired.

7. What science and/or technologies have been transferred and to whom? When is the science and/or technology likely to become available to the end user (industry farmer, other scientists)? What are the constraints, if known, to the adoption and durability of the technology products

Information about the nutritional and clinical importance of status indicators for zinc, copper and magnesium as it becomes available is routinely transferred to a variety of customers.

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National Program(s): 107 100%

The customers include the public through web pages of professional organizations and the Grand Forks Human Nutrition Research Center, and via the popular media, and other scientists through presentations at national and international meetings and professional publications.

8. List your most important publicatons in the popular press (no abstracts) and presentations to nonscientific organizations and articles written about your work (NOTE: this does not replace your peer-reviewed publications which are listed below)

Tracking of research findings cited in periodicals is not routinely done, but it was observed that the finding that high fructose intakes, such as that with a high consumption of soda pop and sport drinks, adversely affects calcium metabolism and thus bone was cited in several magazines and health newsletters including Bottom Line Personal and Remedy.

9. Scientific Publications:

01. Milne, D.B. and Nielsen, F.H. The interaction between dietary fructose and magnesium adversely affects macromineral homeostasis in men. Journal of the American College of Nutrition. 2000. v. 19. p. 31-37.

02. Milne, D.B. Laboratory assessment of trace element and mineral status. IN: J.D. Bogden and L.M. Klevay (eds.) Clinical Nutrition of the Essential Trace Elements and Minerals - The Guide for Health Professionals, Humana Press, Totowa, NJ. 2000. p. 59-90.

Approved: FORREST H NIELSEN Date: 09/00
Title: CENTER DIRECTOR

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0403042 Year: 00 Project Number: 5450-51530-005-00 D
Mode Code: 5450-10-00 STP Codes: 5.1.3.3 50% 5.3.1.1 50%
National Program(s): 107 100%

Title: BIOMARKERS FOR ASSESMENT OF HUMAN MINERAL NUTRITIONAL STATUS AND REQUIREMENTS

Period Covered From: 10/99 To: 09/00

Would you like to terminate this Project? N

Will this project terminate within the first 2 months of FY2001? N

Progress and Outcomes:
1. What major problem or issue is being resolved and how are you resolving it?

Osteoporosis is a common bone disease whose incidence has not been effectively reduced. One out of every two women and one out of eight men over age 50 will have an osteoporosis-related fracture in their life time. Nutritional factors are being recognized as being of particular importance in the prevention of osteoporosis because they can be modified (unlike other risk factors such as genetic predisposition, age, gender). It is generally agreed that calcium and vitamin D are important nutrients for bone health, and supplements containing these nutrients are prescribed widely. However, much less is known about the contribution of suboptimal intakes of other minerals known to be essential for bone development and maintenance (for example, zinc, copper, magnesium) to the incidence of osteoporosis. Also, the effects of large doses of calcium, as found in supplements, on the absorption and utilization of other minerals important to bone health have not been sufficiently determined. Moreover, the effects of common dietary practices like consuming diets high in soy protein versus animal protein on bone metabolism need further elucidation.

As we age, the circulating concentration of an important hormone called insulin-like growth factor-1 (IGF-1) also decreases leading to reduced bone formation and gradual bone loss (this is termed somatopause). It is known that food intake is one of the primary regulators of this bone-building hormone, but the specific effects of varying intakes of trace minerals like zinc, copper and magnesium on this hormone have not been ascertained. Investigations on the effects of different trace minerals on IGF are a new promising area of the potential role of nutrition in prevention of osteoporosis. A series of animal experiments will be conducted to determine how varying intakes of key minerals (e.g., calcium, zinc, copper, magnesium) affect bone health and the IGF proteins. Ovarioectomized rats will be used to simulate postmenopausal state. In addition to the classical measurements of bone mineral content, density, and mechanical properties (e.g.,

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National Program(s): 107 100%

breaking strength) we will monitor changes in bone proteins. The human studies will include calcium supplementation trials with or without other important minerals (zinc, copper, magnesium), and controlled feeding studies to determine the effects of common dietary practices such as eating foods high in soy protein on bone health and to determine if calcium supplementation affects the status of other important minerals in the body. As a part of these studies we will determine how people with different genetic makeup (example difference in vitamin D absorption) respond to calcium supplementation.

2. How serious is the problem? Why does it matter?

The annual cost of healthcare related to osteoporosis is estimated at $14 billion. As the US population ages, the economic cost of osteoporosis is projected to reach $50 billion by the year 2040. It has been estimated that the lifetime risk of fracture exceeds 40% for women and 13% for men. In the elderly, hip fractures are associated with mortality in up to 20% of the cases, with costly long-term nursing home care required for most survivors. Dietary modification is a sensible, practical and economically feasible approach to the prevention of osteoporosis. As trace minerals are known to be essential for bone health, it is important that we determine their specific role in prevention of this devastating disease.

3. How does it relate to the National Program(s) and National Program Component(s)?

This research relates to the National Program 107, Human Nutrition. Specifically, it addresses nutrient requirements, mechanisms of action of nutrients, identification of biomarkers (for the study of bone status), nutrient-nutrient and nutrient-gene interactions, identification of health promoting properties of plant and animal foods, and health promoting intervention strategies for targeted populations.

4. What were the most significant accomplishments this past year?

This project was just initiated; there are no accomplishments to report at this time.

5. Describe the major accomplishments over the life of the project, including their predicted or actual impact.

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National Program(s): 107 100%

This project was just initiated; there are no accomplishments to report at this time.

6. What do you expect to accomplish, year by year, over the next 3 years?

FY 2001: A controlled feeding study will be conducted to compare the effects of consuming soy protein versus animal protein on calcium and bone status of postmenopausal women. A double-blind placebo-controlled supplementation study will be initiated to compare the effects of calcium supplementation with and without other important minerals (specifically zinc, copper and magnesium) on bone loss in postmenopausal women. An animal study will be conducted to examine the effects of consuming marginal amounts of calcium and varying amounts of copper and zinc on bone status and IGF proteins in rats.

FY 2002: The 3-year calcium supplementation trial will continue. The effects of short-term calcium supplementation on trace minerals and calcium retention will be tested in humans. A human study testing the effects of long-term calcium supplementation on trace minerals and calcium retention will be initiated. As genetic makeup plays a critical role in determining bone health, the participants in all the supplementation trials will be genotyped with respect to their vitamin D metabolism. Follow-up animal studies will be designed to expand the findings from previous studies on the effects of trace minerals on bone status and IGF proteins.

FY 2003: The 3-year supplementation trial will continue. The human study examining the effects of long-term calcium supplementation on trace minerals and calcium retention will be completed. Animal studies will continue to expand our findings from previous studies on the effects of trace minerals on bone status and IGF proteins.

7. What science and/or technologies have been transferred and to whom? When is the science and/or technology likely to become available to the end user (industry farmer, other scientists)? What are the constraints, if known, to the adoption and durability of the technology products

None

8. List your most important publicatons in the popular press (no abstracts) and presentations to nonscientific organizations and articles written about your work (NOTE: this does not replace your peer-reviewed publications which are listed below)

None

9. Scientific Publications:

Approved: FORREST H NIELSEN Date: 09/00
Title: CENTER DIRECTOR

MINERAL NUTRIENT FUNCTIONS

MANAGEMENT UNIT

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0400186 Year: 00 Project Number: 5450-51000-018-00 D
Mode Code: 5450-20-00 STP Codes: 5.1.3.3 75% 5.1.3.4 25%
National Program(s): 107 100%

Title: DIETARY TRACE ELEMENTS AND PHYSIOLOGY OF THE CARDIOVASCULAR AND RELATED SYSTEMS

Period Covered From: 10/99 To: 09/00

Would you like to terminate this Project? N

Will this project terminate within the first 2 months of FY2001? N

Progress and Outcomes:

1. What major problem or issue is being resolved and how are you resolving it?

Dietary copper deficiency causes biochemical deficits and structural damage to the cardiovascular system (heart and circulation). Although progress is being made in identifying defects in cardiovascular physiology (function) in copper deficiency, the mechanisms for these functional changes are not completely clear. The known functional changes are not related in a clear fashion to the known structural and biochemical changes. Additionally, because most of the studies on dietary copper have not been done in humans or with intakes consistent with human consumption, the relationship of such findings to human health is not clear. The approaches to resolving this problem include:

A. Determination of functional changes in blood vessels and, in particular, clarification of changes in signal transduction pathways in smooth muscle and endothelium caused by dietary copper deficiency. Relevant studies will be performed with isolated vessels, on isolated organs and on whole animals. The ultimate goal is to determine the contribution of adequate copper nutrition to maintenance of blood flow to organs and to maintenance of blood pressure.

B. Identification of functional changes in the heart and their relationship to metabolic and biochemical alterations caused by trace element (copper) deficiencies. The focus will be to determine coronary blood vessel and cardiac muscle vulnerability to physiologic and metabolic stressors including, but not limited to, adrenaline stimulation and simulated heart attack (cessation and re-starting of blood flow to the heart). Isolated heart and whole animal models will be used.

C. Elucidation of general biochemical mechanisms of damage caused by copper deficiency. Oxidative stress continues to be a strong, although somewhat equivocal, candidate as a mechanism for generalized damage. Damage by oxidative mechanisms will be compared with that caused by another mechanism, glycation. The aim is to attempt to relate known enzymatic, metabolic and hormonal changes to the deterioration of function that occurs in copper deficiency. Various organs will be tested, but the primary focus will be on the heart and blood.

2. How serious is the problem? Why does it matter?

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National Program(s): 107 100%

Studies to date have indicated that dietary copper deficiency has considerable potential for contributing to chronic disease (for example, ischemic heart disease, atherosclerosis, high blood pressure) and the debilitating effects of aging. Experimental evidence indicates that a third or more of the American population may be consuming less than the Estimated Safe and Adequate Dietary Intake of copper set by the Food and Nutrition Board of the National Academy of Sciences. Research will provide information that will be used to set recommendations for dietary copper based on a reduction in risk of chronic disease, particularly of the heart and blood vessels.

3. How does it relate to the National Program(s) and National Program Component(s)?

These studies directly contribute to National Program 107 (100%). Relative to the new Human Nutrition Action Plan, these studies will address elements of Performance Goal 3.1.1 Human nutrition requirements, with specific emphasis on identifying potentially beneficial mechanisms of action of trace elements and characterizing the role of trace elements in achieving optimal physiologic function.

4. What were the most significant accomplishments this past year?

A. Single most significant accomplishment during FY 2000:

Individual blood vessels from copper-deficient animals dilate poorly when dilation requires the release of a specific chemical (nitric oxide) within the blood vessel. To determine whether this impairment alters blood pressure in copper-deficient animals, we examined the effect of an agent that blocks nitric oxide release on blood pressure in copper-adequate and copper-deficient rats. The blocker caused a higher increase in blood pressure in copper-adequate than in copper-deficient animals. This demonstrates that dietary copper deficiency alters the regulation of blood pressure and further indicates the dependence of good cardiovascular health upon adequate dietary copper.

B. Other significant accomplishments:

Severe dietary copper deficiency impairs the ability of the heart to contract and use energy properly. To determine whether this impaired heart function leads to heart failure similar to that caused by other conditions (such as high blood pressure), genetic expression of certain proteins that are indicative of heart failure and indices of cell viability were measured in heart cells from copper-deficient mice. Genes for proteins that are only found in failing hearts were elevated and a phenomenon called programmed cell death was found to be increased in hearts from copper-deficient mice. These findings support the view that dietary copper deficiency can lead to heart failure and that good cardiovascular health depends upon adequate dietary copper. (Collaborator - Y.J. Kang,

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National Program(s): 107 100%

University of Louisville).

Because hearts from copper-deficient animals show impaired contractile and metabolic function, it is important to understand this impairment on a cellular level. Individual cells from copper-adequate and copper-deficient hearts were examined microscopically for their ability to contract and relax (shorten and lengthen)and to observe the movement of calcium (which is necessary for contraction) within the cell. Cells from copper-deficient hearts contracted faster and more forcefully and showed stronger and faster calcium movements than those of copper-adequate hearts. This apparent enhancement of heart cell function in the face of impairment of function of the whole heart suggests that some aspects of function compensate for a decline in others and that further study is important to the complete understanding of the effects of copper deficiency on the heart. (Collaborator - J. Ren, University of North Dakota).

5. Describe the major accomplishments over the life of the project, including their predicted or actual impact.

We have shown that the concentrations of two important chemical messengers, nitric oxide and cyclic GMP, are elevated in copper-deficient hearts, which suggests their possible role in reduction of heart contractile force in copper deficiency. A subsequent study showed that the amount of one of the enzymes that produces nitric oxide, inducible nitric oxide synthase, is also elevated in copper deficiency, which suggests an elevated genetic expression of this enzyme. These studies help to define the molecular basis for impaired heart function when dietary copper is restricted.

Strong evidence was found supporting the view that glycation, the undesirable binding of sugar to proteins, is enhanced in dietary copper deficiency. Blood analysis revealed the presence of glycated hemoglobin and fructosamine (blood proteins with sugar bound to them) as well as pentosidine (a product of blood protein damaged by glycation). Because glycation is a process that is increased in diabetes and aging, this finding suggests that reduced copper intake may worsen the consequences of these two conditions.

Measurements of heart and blood vessel function in copper deficient animals helped to show that, although cardiac output was not altered by copper deficiency, blood vessel resistance was reduced and volume of blood ejected per beat (stroke volume) of the heart was elevated; the higher stroke volume may contribute to the pathologically greater size of copper-deficient hearts. These and succeeding physiological measurements will help to characterize heart function in dietary copper deficiency. Studies with collaborators on the effect of copper deficiency on blood clotting function have shown that the aggregation of blood platelets to one another was increased and that adhesion of platelets to blood vessel

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National Program(s): 107 100%

endothelial cells was reduced. Further, these findings were associated with an alteration of two platelet clotting factors, fibrinogen and von Willebrand factor. These studies emphasize the importance of dietary copper to prevent bleeding.

Another collaborative study found that the dilation of blood vessels in response to an inflammatory agent was exaggerated in copper-deficient rats. By use of appropriate blocking agents, the potential mechanism(s) responsible for this change were delineated. This study shows the importance of proper copper intake in mediating the body's inflammatory response to injury.

6. What do you expect to accomplish, year by year, over the next 3 years?

FY2001: We will examine whether and to what extent the nitric oxide signal transduction pathway is involved in the altered function of copper-deficient hearts; this will include examination of nitric oxide effects on contractile function and mitochondrial respiration as well as cardiac genetic expression of the enzymes that produce nitric oxide in copper-deficient hearts. FY2002: Research will be directed toward determining whether a direct association can be made between formation of advanced glycation end-products and altered cardiovascular function of copper deficiency; examination of heart and blood vessel function will be made in the presence of known inhibitors of glycation. FY2003: Because a positive finding in the latter study would suggest altered carbohydrate metabolism, we will examine the role of the pancreas in initiating cardiovascular effects of dietary copper deficiency; this would include examination of the role of nitric oxide on pancreatic function (i.e., hormone release)and whether the resulting alteration of carbohydrate metabolism could be shown, by functional testing, to account for cardiovascular effects.

7. What science and/or technologies have been transferred and to whom? When is the science and/or technology likely to become available to the end user (industry farmer, other scientists)? What are the constraints, if known, to the adoption and durability of the technology products

Interviewed by USDA Radio on the subject of copper and aging, January, 2000.

Interviewed on the subject of copper deficiency, glycation and aging by writers from Prevention, Great Life and Life Extension magazines and the New York Daily News, January, 2000.

Seminar entitled "Copper Deficiency and the Cardiovascular System" was presented to the GFHNRC staff, January 13, 2000.

Information was transferred to the public through an article in the nutrition section of The Grand Forks Herald newspaper entitled "Preventing free radical damage will slow aging - Vitamins and minerals play a key role in determining how long we live," May, 2000.

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8. List your most important publicatons in the popular press (no abstracts) and presentations to nonscientific organizations and articles written about your work (NOTE: this does not replace your peer-reviewed publications which are listed below)

Studies on the relationship between copper deficiency, glycation and aging were cited in USDA-ARS publication Food and Nutrition Research Briefs, October, 1999.

Research on glycation and aging was cited in Prevention magazine, June, 2000.

9. Scientific Publications:

01. Saari, J.T., Schuschke, D.A. Cardiovascular effects of dietary copper deficiency. BioFactors. 1999. v. 10(4). p. 359-375.

02. Schuschke, D.A., Percival, S.S., Saari, J.T., Miller, F.N. Relationship between dietary copper concentration and acetylcholine-induced vasodilation in the microcirculation of rats. BioFactors. 1999. v. 10(4). p. 321-327.

03. Kang, Y.J., Wu, H., Saari, J.T. Activation of hypertrophic gene expression by dietary copper restriction in mouse heart. Proceedings of the Society for Experimental Biology and Medicine. 2000. v. 223(3). p. 282-287.

04. Kang, Y.J., Zhou, Z-X., Wu, H., Wang, G-W., Saari, J.T., Klein, J.B. Metallothionein inhibits myocardial apoptosis in copper-deficient mice: Role of atrial natriuretic peptide. Laboratory Investigation. 2000. v. 80(5). p. 745-757.

05. Schuschke, D.A., Falcone, J.C., Saari, J.T., Fleming, J.T., Percival, S.S., Young, S.A., Pass, J.M., Miller, F.N. Endothelial cell calcium mobilization to acetylcholine is attenuated in copper-deficient rats. Endothelium. 2000. v. 7(2). p. 83-92.

06. Saari, J.T. Dietary copper deficiency reduces elevation of blood pressure caused by nitric oxide synthase inhibition. The FASEB Journal. 2000. v. 14(4). Abstract p. A773.

07. Wold, L.E., Saari, J.T., Ren, J. Isolated ventricular myocytes from copper-deficient rat hearts exhibit enhanced cardiac contractile function. The FASEB Journal. 2000. v. 14(4). Abstract p. A157.

08. Wold, L.E., Saari, J.T., Ren, J. Isolated ventricular myocytes from copper-deficient rat hearts exhibit enhanced cardiac contractile function. 2nd Biennial Joint EPSCoR Conference Abstracts. 1999. p. 29.

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Publications: (Continued)

09. Saari, J.T. "Copper and cardiovascular disease" presented at the Department of Pharmacology, Physiology and Therapeutics, University of North Dakota, November 1999.

10. Saari, J.T. "Copper deficiency and the cardiovascular system: Role of peroxidation, glycation and nitration" presented at the Department of Anatomy and Cell Biology, University of North Dakota, Grand Forks, ND, January 2000.

11. Saari, J.T. "Dietary copper deficiency reduces elevation of blood pressure caused by nitric oxide synthase inhibition" presented at the Experimental Biology 2000 meeting, San Diego, CA, April 2000.

Approved: FORREST H NIELSEN Date: 09/00
Title: CENTER DIRECTOR

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Title: MINERAL ELEMENT NUTRITION, NEUROPSYCHOLOGICAL FUNCTION AND BEHAVIOR

Period Covered From: 10/99 To: 09/00

Would you like to terminate this Project? N

Will this project terminate within the first 2 months of FY2001? N

Progress and Outcomes:

1. What major problem or issue is being resolved and how are you resolving it?

The consequences of mild to moderate mineral deficiencies for neuropsychological function and behavior are largely unknown. This represents a serious problem because suboptimal mineral intakes and status have been linked to chronic disorders such as depression and dementia. Further, national nutrition surveys indicate that dietary intakes of several essential minerals are less than recommended in many segments of the U.S. population and existing data are frequently inadequate or unavailable to make recommendations based on functional outcomes. This project addresses the need for increased experimentally derived knowledge leading to a better understanding of the relationships among mineral element nutrition, neuropsychological function and behavior. Such knowledge is critical when making recommendations for mineral intakes that will facilitate optimal neuropsychological health and performance throughout the life span in all segments of our population. Behavior is unique as a criterion for establishing nutritional adequacy because it represents the functional integration of all biological systems, including compensatory mechanisms that often determine the practical importance of a nutritional deficit or excess.

Neuropsychological and behavioral consequences of mild and moderate deficiencies in biologically essential mineral elements are determined with the goal of improving health, work and school performance, and sense of well-being in the population. Specifically, studies are designed to determine: the role of mineral elements in cognition (i.e., attention, perception, learning, memory and reasoning) and spatial and motor skills; the effect of mineral nutrition on mood states and emotional and social adjustment; the impact on nutrition-behavior relationships of potential mediating factors, including environmental and endogenous stressors like noise, temperature, sleep duration and quality, and menstrual and menopausal symptoms; and, the effect of mineral nutrition on electrophysiology indexing brain function to gain insights into the mechanisms for nutritional effects on performance and sense of well-being. New methods and technologies are developed to increase efficacy of behavioral assessments and promote their use by other nutrition scientists. Studies of healthy adults and children are complemented by animal studies.

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2. How serious is the problem? Why does it matter?

Findings obtained during the past 40 years indicate that the mineral elements boron, copper, iodine, iron, magnesium, manganese, selenium and zinc likely are important for normal neuropsychological function and behavior of adults and children. However, previous studies have yet to establish the reliability of effects of graded mineral intakes on behavior or to adequately characterize the relationship between mineral element nutrition and brain function and cognition. Such information is critical to characterizing the mechanisms responsible and is needed to apply findings to real-world problems. To respond to intense public interest in the relationship between nutrition and performance, and potentially to improve public health, productivity and sense of well-being, there is a great need to increase our knowledge of the functional consequences of graded intakes of mineral elements, and especially the consequences of marginal intakes common in many segments of the population. Food consumption surveys indicate that intakes of calcium, copper, iron, magnesium and zinc are significantly below the RDA or ESADDI for large segments of the adult population in the United States and worldwide, and many reviews have concluded that mild-to-marginal deficiencies in these and other mineral elements are particularly likely in the groups targeted by this research (e.g., women, children, elderly). Further, increased knowledge of the relationship between mineral element nutrition, neuropsychological function and behavior is needed for a more complete determination of nutrient requirements, establishing recommended dietary intakes, and evaluating the efficacy and adverse effects of taking dietary supplements, a multi-billion dollar industry in the United States.

3. How does it relate to the National Program(s) and National Program Component(s)?

This research relates to program 107, Human Nutrition (100%). The program component most directly addressed by this project is Nutrient Requirements, with objectives including identifying and developing indicators of function and performance that are sensitive to changes in nutrients and bioactive components of the diet, and characterizing the role of nutrients and other dietary components in achieving and maintaining optimal physiologic and psychologic function and performance. This research also addresses the objectives to determine the functional impact of interactions among dietary constituents and among nutrients and lifestyle, environmental and genetic factors. Products of this research also relate to the program components, Relationship between Diet, Genetics and Lifestyle and the Risk for Chronic Disease, and Health Promoting Intervention Strategies for Targeted Populations.

The determination of dietary requirements for optimal cognitive function and performance has been identified as a national need. This

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research furthers ARS objectives by directly evaluating, under highly controlled conditions, the effects of mineral element nutrition on neuropsychological function and behavior of adults, including the elderly, and in children and adolescents. This research facilitates the detection of mild mineral deficiencies and helps define dietary mineral requirements to develop and maintain health and optimal function throughout the life cycle. Examining the combined effects of nutritional insults and exogenous and endogenous stressors offers insights into ways to improve performance in work and school, and in other situations with a high demand. Understanding the true role of mineral element nutrition in neuropsychological function and behavior also helps individuals and groups to more knowledgeably evaluate nutrition claims, and promotes healthy and cost-efficient dietary behavior.

4. What were the most significant accomplishments this past year?

A. Single Most Significant Accomplishment during FY 2000:

While it is known that zinc (Zn) is essential for growth and brain function during early development, and there is evidence from this laboratory showing the importance of Zn for cognitive function of school-aged children, prior research has not examined the possible relationship between Zn nutrition and mood and behavior problems in children. In collaboration with investigators from the University of Texas Medical Branch at Galveston, University of Texas at Brownsville, COPRIMA, and the Brownsville Independent Public School District, and with funding from the USDA CSREES National Research Initiative and Gerber Foundation, we assessed zinc status and adaptive functioning, behavior problems and school performance of children (aged 6-9 years) living in Brownsville, TX and at risk for Zn deficiency because of life stage and regular consumption of cultural foods high in phytate. The study found that hair Zn concentration was negatively associated with teacher-reported anxiety and depression, withdrawal and total adjustment problems, indicating that problems associated with lower Zn status were personality rather than conduct in nature. Findings provide the first evidence that Zn status is predictive of mood disturbances and behavior problems in school-aged children, and point to the need for further study to determine whether increased Zn intakes might prevent or help alleviate mood and behavior problems that directly affect school performance, cognitive and social development, and quality of life for many children.

B. Other Significant Accomplishments:

Vitamin B12 deficiency is known to cause cognitive impairment in the elderly, but despite a relatively high incidence of B12 deficiency among children in many parts of the world, no research has examined the relationship between B12 nutrition, cognition and behavior in children. Biochemical indices of B12 status were determined in at-risk Guatemalan school children (aged 8-12 years) and statistically related to measures of adaptive functioning, behavior problems and school performance.

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Methylmalonic acid and homocysteine indicated a positive relationship between B12 status and academic performance and adaptive function, and a negative relationship between B12 status and attention problems, while folate indicated a negative relationship between B12 status and withdrawal, somatic complaints and social problems. These behavioral effects complement findings that children with low and marginal B12 status also performed less well on computerized neuropsychological tests to provide the first evidence that B12 deficiency is associated with cognitive and behavioral impairment in children as well as in the elderly.

Although exposure to high concentrations of manganese (Mn) in air and contaminated water result in many neurologic disorders, toxic effects of high Mn intakes from food are unknown and regulatory guidelines such as the current Reference Dose of 10 mg/day for an average adult male appear to conflict with the estimated Mn intakes of vegetarians of 10-17 mg/day. Healthy premenopausal women were fed either 1 or 20 mg/day manganese for 8 weeks as part of a highly controlled metabolic unit study and examined double-blind by a licensed neurologist for the occurrence and severity of more than 40 neurologic signs and symptoms. Neither dietary group showed any signs or symptoms. This finding indicates that dietary intakes in the range consumed by many vegetarians are unlikely to result in neurologic problems and underscores the need to coordinate efforts to establish recommended dietary intakes and to set regulatory guidelines to protect the population from toxicologic effects of minerals naturally found in the diet.

5. Describe the major accomplishments over the life of the project, including their predicted or actual impact.

Demonstrated that low copper (Cu) intake (1 versus 3 mg/day) can impair short-term verbal memory of healthy postmenopausal women, indicated by increased intrusions during verbal recall and finding that Cu status indicators (plasma Cu and ceruloplasmin) were positively correlated with and predicted improved verbal memory, improved long-term memory, increased clustering of related verbal material (strategy) and fewer intrusions during recall (reduced distraction). A moderately high zinc (Zn) intake (53 mg/day) combined with a low Cu intake, improved list recall, but impaired verbal span and a general measure of short-term memory. These data increase our understanding of the role of Cu nutriture in cognitive function, which has received little study, and address the interactive effects of moderately high Zn intakes combined with low Cu intakes. The impact of these findings was to spur additional study of the relationship between dietary Cu and cognitive function, including dementia, with the potential impact to affect recommended intakes and improve cognitive function in the elderly and other populations.

Determined that short-term supplementation with zinc (Zn) combined with other micronutrients may improve some aspects of cognitive function

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of school-aged Mexican-American children, who are at increased risk for Zn deficiency primarily because of high intakes of dietary phytate. Children (aged 6-9 years) living in Brownsville, TX were treated with a micronutrient (M) mixture containing 50% RDA or mean ESSADI vitamins and minerals (excluding iron, calcium, magnesium, phosphorus and Zn, with folate at 25% RDA), 20 mg/d Zn plus micronutrients (Zn+M), 20 mg/d iron plus micronutrients, or a placebo (P) for 10 weeks in a double-blind control trial. Compared to the other treatments and P, Zn+M improved reasoning, indicated by fewer number of trials needed to learn simple concepts. This research complements earlier work by this laboratory that found a similar treatment regimen improved cognitive and psychomotor function of Chinese children with a high incidence of Zn deficiency primarily caused by inadequate Zn intakes. Findings provide further indication that Zn supplementation may significantly benefit cognitive function in deficient populations, and eventually may be used in setting recommended intakes for Zn and formulating school meal programs. While it is known that Zn is essential for growth and early development, these data provide evidence that Zn intake is important to cognitive function in later development.

Determined that zinc (Zn) deprivation (4.5 mg/day for 2 months) affected short-term verbal memory of healthy adult men. Reaction times to recognize previously learned words were initially increased and then decreased in response to deprivation, which suggests adaptation to reduced Zn intakes. Numerous other aspects of cognitive function and psychomotor performance were also evaluated, but no evidence was found for any other effects of low dietary Zn intakes in this sample. Findings complement previous research showing that Zn nutriture may affect cognitive function in the adult, provide experimental evidence that homeostatic controls may compensate for short-term cognitive effects during chronic Zn undernutrition, and indicate that memory is the cognitive process most sensitive to Zn deprivation. Findings help other scientists focus the goals and approach of future studies of Zn and cognitive function.

Determined that zinc (Zn) and micronutrient supplements improved cognitive and psychomotor function of rural and urban Chinese school children. Motor skills, including manual dexterity and eye-hand coordination, visual perception, memory for simple shapes and complex objects, and reasoning were functions most affected by Zn (20 mg/day) and Zn combined with other micronutrients at 50% recommended intakes. Findings indicate that cognitive and psychomotor function, and thus school performance, may be suboptimal in the >25% of Chinese children and 6-10% of school-aged children in the United States who are Zn deficient. This was the first evidence that Zn supplementation of young children may improve cognition and psychomotor function. Findings were widely reported by the national media, followed by many inquiries from the public, private industry and granting agencies, and by invitations to speak on this research. This research led directly to collaborative studies of cognitive and psychomotor effects of Zn supplementation of Mexican-American school

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children (supported by grants from the Gerber Foundation and USDA National Research Initiative), of zinc and iron supplementation of young women (supported by grant from the US Army Medical Research, Development, Acquisitions and Logistics Command), and of Zn deprivation of young adult men (supported by USDA ARS Western Human Nutrition Research Center).

Experimentally determined that adult male rats fed diets deficient (1 ug/g diet) or excessive (100 ug/g diet) in manganese (Mn) were generally less active than those fed adequate Mn (10 ug/g diet), and consequently, these rats engaged in fewer aggressive behaviors (attacking, biting, wrestling, aggressive contact) and displacement activities (exploration, self-grooming), and more posturing. However, rats fed diets high in Mn but low in calcium (Ca; 2500 versus 5000 ug/g diet) did show increased aggressive behavior compared to rats fed other diets. Findings provide weak support for earlier reports that Mn excess increases aggression, primarily because rats fed high Mn were less active than those fed adequate Mn. No support was found for the hypothesis that Mn deprivation is associated with increased aggression. Mn is essential for normal brain function and behavior, and there has been speculation that moderate Mn intoxication or deficiency may be associated with increases in aggressive behavior. Findings from this study expand knowledge of the functional role of dietary Mn, and its interaction with Ca, at physiological as well as toxicological concentrations. Predicted impact is redirection of future research on possible nutritional involvement in aggressive behavior to study the interaction of mineral nutrients.

Showed that dietary selenium (Se) affects mood states of healthy adults. Men in the United States with typically adequate Se intakes and fed approximately 3 times the RDA for Se for 12 weeks reported less depression and mental confusion than men fed approximately one-third the RDA. Women in New Zealand with typically low Se intakes and supplemented daily with 40 ug Se reported more energy and confidence, less hostility, and a decrease in total mood disturbance after 15 weeks. Findings indicate a novel function for dietary Se that may be used to help establish Se requirements for adults. Findings were widely reported by the national media, followed by numerous inquiries from other researchers and the general public. Extension of this research to assess cognitive performance and brain electrical activity in response to changes in Se intakes is a component of a recently awarded grant from the USDA CSREES National Research Initiative Program to study the efficacy of feeding Se-enhanced meat, wheat and broccoli to healthy adults in the Peoples Republic of China.

Determined in mature rats that low dietary intake for 10 weeks of either copper (Cu) (0.05 versus 6.0 ug/g) and magnesium (Mg) (50 versus 500 ug/g) was associated with an increase in generalized activity. Low Cu intake also resulted in more stereotypic behavior during presentation of an auditory stressor, whereas low Mg intake resulted in increased stereotypic behavior regardless of the presence of the stressor. Low Cu intake was associated with poorer performance on measures of learning,

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whereas low Mg intake was associated with poorer performance on measures of memory. Neither Cu nor Mg showed strong effects on direct measures of anxiety; however, indirect measures of stressor effects during activity monitoring and memory testing suggest that both minerals may impact emotionality. Cu and Mg are two minerals of potential relevance to behavior because of their importance in neurotransmitter metabolism and because previous studies have shown that dietary intakes of both minerals affect brain electrophysiology. Findings indicate that both Cu and Mg have functional consequences at the behavioral (and possibly emotional) level, which complement earlier findings of effects of these two minerals on brain physiology. Predicted impact is increased efficacy of future research on neuropsychological function and behavior of humans.

6. What do you expect to accomplish, year by year, over the next 3 years?

Expected Accomplishments during FY 2001:

Plan and initiate a field study of the relationship between zinc intakes and status, body composition, cognitive and adaptive function, and psychoeducational performance in children, aged 12-14 years, attending local middle schools. Zinc intakes will be manipulated by food fortification or supplementation. A new mobile research laboratory will be used to make all assessments. Findings will help determine needed zinc intakes to promote optimal physical and behavioral development of adolescents at-risk for zinc deficiency because of rapid growth.

Determine the individual and combined effects of dietary copper and zinc intakes on cognitive function, particularly memory, and brain electrophysiology of healthy postmenopausal women participating in a controlled metabolic unit study. Findings will help determine needed intakes of copper and zinc for optimal cognitive and brain function in older individuals, and to verify previously observed interactive effects where moderately high zinc intakes impaired some aspects of memory in individuals with low copper intakes.

Implement a study to determine in mature rats the interactive effects of altered glucocorticoid concentrations and dietary zinc intakes on hippocampal damage and working and reference memory, activity and emotionality. Previous studies in rats indicate that environmental and other stressors increase glucocorticoid concentration in blood, damage cells in the hippocampal region of the brain and may impair memory. Zinc deficiency has also been linked to hippocampal damage, memory impairments and hyperemotionality. Findings will indicate whether zinc deficiency increases risk of memory impairments in response to stress and, conversely, whether adequate zinc intakes protect against such impairments.

Determine the relative efficacy of different biochemical forms of selenium supplementation on central nervous system function and behavior in mature rats. Findings will facilitate future research with humans and may be used to help determine needed intakes of selenium to achieve

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optimal performance.

During FY 2002: Determine the effects of dietary magnesium intakes on brain electrophysiology, sleep quantity and quality, and activity levels of healthy postmenopausal women participating in a controlled metabolic unit study. Findings will help determine whether magnesium has a role in maintenance of normal sleep patterns and the mitigation of symptoms associated with sleep disorders and deprivation.

Implement a field study of the relationship between zinc intakes and status, body composition, cognitive and adaptive function, and psychoeducational performance in children, aged 12-14 years, attending local middle schools. See FY 2001 above for impact.

Plan and implement an intervention study with Guatemalan children aged 6-9 years to determine whether supplementation or food fortification with vitamin B12 will improve cognitive and psychomotor skills, adaptive function and school performance. This research will be a follow-up to a recently completed study showing a correlation between B12 status and these behavioral measures in this at-risk population.

Plan and initiate a field study to determine the nutritional and health status of local institutionalized and non-institutionalized elderly. Nutritional assessment will focus on mineral nutrition, particularly zinc and magnesium. Brain and cognitive function, mood states, sleep quantity and quality, and physical activity will also be measured and related to nutritional status. Findings will be used to facilitate future intervention studies with this at-risk population.

During FY 2003: Plan and initiate an epidemiological study to determine the relationship between mineral element nutrition, incidence of disease, physiologic and psychological function and behavior in Native Americans served by the Aberdeen Area Indian Health Services. A mobile research laboratory will be used to make all assessments. Findings will facilitate future intervention studies with this at-risk population.

Determine the relative benefits for cognitive function of feeding selenium-enhanced meat, wheat and broccoli to adults in the Peoples Republic of China. This research is scheduled as part of a recently funded, long-term project on selenium bioavailability and function.

Determine the efficacy of copper and iron supplementation for sleep quantity and quality in healthy young adults living in the local community, and for relief of depression in clinically diagnosed institutionalized and free-living adults. Results will validate previous findings of a relationship between status indicators of these minerals and sleep morphology and depressive symptoms.

7. What science and/or technologies have been transferred and to whom? When is the science and/or technology likely to become available to the end user (industry farmer, other scientists)? What are the constraints, if known, to the adoption and durability of the technology products

As member of the DRI Panel on Micronutrients (Food and Nutrition

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Board, Institute of Medicine, National Academy of Sciences), reviewed and interpreted scientific literature and expert testimony on arsenic, boron, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium and zinc nutrition for determination of the new Dietary Reference Intakes for the U.S. population. Contributed to the formulation of DRIs and preparation of draft report submitted for NAS review, with an expected release date of December 2000.

Presented the framework underlying the new Dietary Reference Intakes to nutrition professionals in an invited address to the Greater Grand Forks Dietetics Association, Grand Forks ND, March 2000, and described the differences between the new and previous dietary recommendations to the lay public in a newspaper column, Watch Your RDAs and Your DRIs!, Grand Forks Herald, December 29, 1999, p. B1.

To meet the need for valid yet inexpensive and easy-to-use procedures to routinely assess the relationship between nutrition and behavior, a computer software package and associated procedures were developed to automate the administration of standardized neuropsychological tasks designed to assess a variety of cognitive processes (e.g., perception, attention, learning, memory and reasoning) and psychomotor and spatial skills. Initially designed for English speaking adults, tasks and instructions have recently been redesigned to be more image-based for use with children and non-English speaking persons. This technology and related methods represent significant contributions to research on the neuropsychological and behavioral effects of nutritional deficiencies and supplementation. Users of this technology are researchers in private industry and in state and federal governments, domestic and foreign. Currently in progress are 5 collaborative research projects using this technology, supported by 4 granting agencies, and involving 5 principal investigators in 3 countries.

Continued updates and enhancements of this technology will ensure its durability. Lack of familiarity with behavioral and computerized testing, the need for careful training of test administrators, and the lack of age- and country-specific norms are current constraints on adoption of this technology.

Presentations of this research program and recent findings were made to classes from the Food and Nutrition and Chemistry departments at Concordia College, MN (11/18/99), the Vitamins and Minerals class from ND State University (4/20/2000), and to teachers from New Folden (MN) High School (6/5/2000).

8. List your most important publicatons in the popular press (no abstracts) and presentations to nonscientific organizations and articles written about your work (NOTE: this does not replace your peer-reviewed publications which are listed below)

Presentation to Nonscientific Group:
Presentation of this research program and recent findings was made to

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the alumni from the University of North Dakota (5/24/2000).

Media Coverage:

Nutrition Studies Get Psyched Over the Internet. Agricultural Research. Nov 1999. p. 16-17.

This research program, including study results, also receives public exposure through frequent coverage by the popular press (local, regional and national newspapers, magazines, radio and television) and industry newsletters and magazine.

9. Scientific Publications:

01. Penland, J.G. Behavioral data and methodology issues in studies of zinc nutrition in humans. Journal of Nutrition. 2000. v. 130(Suppl.2). p. 361S-364S.

02. Lykken, G.I., Ong, H.S., Alkhatib, H.A., Harris, T.R., Momcilovc, B., Penland, J.G. Perquisite spin-off resulting from twenty-two years of measuring background activity in the whole body counter steel room. Annals of the New York Academy of Sciences. 2000. v. 904. p. 267-270.

03. Sandstead, H.H., Frederickson, C.J., Penland, J.G. History of zinc as related to brain function. Journal of Nutrition. Feb 2000. v. 130(Suppl.2). p. 496S-502S.

04. Penland, J.G., Milne, D.B., Davis, C.D. Moderately high zinc intake impairs verbal memory of healthy postmenopausal women on a low copper diet. Roussel, A.M., Favier, A.E., Anderson, R.A., editors. Plenum Publishing, London. Trace Elements in Man and Animals 10. 2000. p. 1025-1030.

05. Kretsch, M.J., Fong, A.K.H., Penland, J.G., Sutherland, B., King, J.C. Cognitive effects of adaptation to a low zinc diet in healthy men. Roussel, A.M., Favier, A.E., Anderson, R.A., editors. Plenum Publishing, London. Trace Elements in Man and Animals 10. 2000. p. 999-1001.

06. Sandstead, H.H., Penland, JG. Zinc and psychomotor development. Roussel, A.M., Favier, A.E., Anderson, R.A., editors. Plenum Publishing, London. Trace Elements in Man and Animals 10. 2000. p. 981-986.

07. Penland, J.G. The new Dietary Reference Intakes (DRIs) for essential minerals, including Tolerable Upper Intake Levels. Neurotoxicology and Teratology. 2000. v. 22. Abstract p. 463.

08. Penland, J.G., Allen, L.H., Boy, E., DeBaessa, Y., Rogers, L.M. Adaptive functioning, behavior problems and school performance of Guatemalan children with deficient, marginal and normal plasma vitamin B12. The FASEB Journal. 2000. v. 14. Abstract p. A561.

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Publications: (Continued)

09. Penland, J.G., Davis, C.D., Finley, J.W., Pettit, R.E. Moderately high dietary intakes of manganese do not cause neurologic signs or symptoms in healthy adult women. The FASEB Journal. 2000. v. 14. Abstract p. A261.

10. Carroll, R.M., Egger, N.G., Alcock, N.W., Dayal, H.H., Penland, J.G., Sandstead, H.H. Effects of Zn or Fe on lean body mass, serum insulin & IGF-1. The FASEB Journal. 2000. v. 14. Abstract p. A729.

11. Finley, J., Davis, C., Penland, J. Interactive effects of dietary fat type and manganese concentration in healthy young women. The FASEB Journal. 2000. v. 14. Abstract p. A297.

12. Penland, J.G. Zinc and neuropsychological function: results of recent intervention trials and implications for future studies. Invited address to the International Symposium on Zinc and Human Health, Micronutrient Initiative and University of California at Davis, Davis CA, October 1999.

13. Penland, J.G. "Adaptive functioning, behavior problems and school performance of Guatemalan children with deficient, marginal and normal plasma vitamin B12" presented at the Experimental Biology 2000 meeting, San Diego, CA, April 2000.

14. Penland, J.G. "Moderately high dietary intakes of manganese do not cause neurologic signs or symptoms in healthy adult women" presented at the Experimental Biology 2000 meeting, San Diego, CA, April 2000.

15. Penland, J.G. The new Dietary Reference Intakes (DRIs) for essential minerals, including upper limits. Invited address to the symposium on Toxic and Essential Trace Elements: Neurobehavioral Assessment, Neurobehavioral Teratology Society, Palm Beach FL, June 2000.

Approved: FORREST H NIELSEN Date: 09/00
Title: CENTER DIRECTOR

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ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0401450 Year: 00 Project Number: 5450-51000-023-00 D
Mode Code: 5450-20-00 STP Codes: 5.1.3.3 80% 5.1.3.4 20%
National Program(s): 107 100%

Title: BIOCHEMICAL CONSEQUENCES OF SUBOPTIMAL DIETARY INTAKE OF TRACE ELEMENTS

Period Covered From: 10/99 To: 09/00

Would you like to terminate this Project? N

Will this project terminate within the first 2 months of FY2001? N

Progress and Outcomes:
1. What major problem or issue is being resolved and how are you resolving it?

Neither the explicit health effects of low dietary intakes of copper, zinc and selenium or the dietary requirements for these elements for health and optimal performance are well-defined. Because zinc and copper serve as cofactors for a large number of enzymes that catalyze important biochemical reactions, the effects of low zinc and copper intakes on the in vitro activities of these enzymes is one paradigm that has been used for estimating human and animal requirements for zinc and copper. Similarly, selenium is important to the activities of several enzymes that protect cells against damaging oxygen radicals and oxidative damage to cell components resulting from low selenoenzyme activities has been implicated in the pathology associated with selenium deficiency. However, the paradigm of reduced enzymatic activities has not provided sufficient information to account for the biological and health consequences of low dietary intakes of copper, zinc and selenium. A major reason for this insufficiency is the lack of knowledge regarding how reductions in the activities of zinc-, copper-, and selenium-dependent enzymes translate into altered cellular and organ function and the lack of knowledge regarding non-enzymatic roles of these elements.

Animal models of copper and zinc deficiencies and cells cultured in media containing various concentrations of zinc, copper, and selenium are used to investigate the various biochemical mechanisms underlying the functional consequences of low zinc and copper intakes and low cellular content of zinc and copper. The influences of copper and zinc deprivation on the synthesis of bioactive molecules, transport mechanisms, transmembrane signaling, mitochondrial function, and mechanisms regulating programmed cell death will be determined. Knowledge based on the descriptions of biochemical mechanisms for the functional outcomes of zinc and copper deficiencies can more precisely define the dietary requirements for zinc and copper for health and optimal performance during all stages of life in men and women.

There are numerous brands of supplements sold in the marketplace that contain large amounts of zinc. At present, these zinc supplements and their contents are not regulated. Certain dietary nutrients such as copper and zinc interact in the gut to inhibit the absorption of each other. However, zinc affects copper absorption and utilization more than copper affects zinc. If a person consumes two to three times the

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National Program(s): 107 100%

requirement (RDA) of zinc, either from food or, more likely, from food supplements for an extended period, copper absorption could be reduced to such an extent that the person may develop a moderate grade copper deficiency. Therefore, there is a need to understand the physiological effects of high zinc intakes on copper absorption and utilization. A human intestinal cell model is used to determine the basic mechanisms involved in the copper-zinc absorption interaction. This information can be used to help set standards for zinc contents in food and food supplements, and for use in making recommendations for copper intake when dietary zinc might be in excess.

Selenium can also interact with other dietary or environmental trace elements. These interactions may interfere with the anticancer function of selenium by impairing its role in triggering the death of cancer cells. A human leukemia cell model will be used to investigate the basic mechanisms through which interactions between selenium and other dietary or environmental trace elements influence programmed cell death. This information will be useful for designing dietary strategies to establish multiple mineral requirements to decrease the risk of cancer.

2. How serious is the problem? Why does it matter?

Current information indicates that copper and zinc intakes for a large portion of the population are below the currently recommended amounts. In particular, lower than recommended copper intakes were found in every age-sex group surveyed in the United States. It is not known if low intakes of zinc and copper can have long-term health effects, particularly in the development of degenerative diseases of the cardiovascular and nervous systems and in fetal development. Knowledge regarding the biochemical mechanisms leading to negative health effects of low zinc and copper intakes can provide a basis for recommending dietary requirements that can slow or ameliorate the development of degenerative diseases. Dietary interventions based on this knowledge could reduce the $200 billion spent yearly in the United States for treatment of diseases that are strongly associated with diet.

More than 80% of the diets consumed in the United States do not contain the recommended allowance for copper. Therefore, an intake of a small excess of zinc in the form of a supplement could easily promote or induce a mild to moderate copper deficiency. Copper is an essential nutrient for myriad physiological and biochemical processes, many of which are involved with development and function of the nervous system in the fetus. Although zinc is also required for development, it is extremely important to know the precise balance between the two nutrients so that an excess of one can be counterbalanced by the proper intake of the other.

Epidemological and human studies have revealed that selenium is an effective anticancer agent. However, interactions between arsenic and selenium could reduce the anticancer properties of selenium. It has been suggested that the cancer risk from arsenic in the drinking water of the

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United States is comparable to that of environmental smoke and radon in homes. Thus, it is important to understand how the interaction between selenium and arsenic or other elements affects the mechanism involved in initiating and regulating cell death. Such information will help establish dietary selenium requirements for optimizing the anticancer effects of selenium in relation to dietary intakes of arsenic and other elements that interact with selenium.

3. How does it relate to the National Program(s) and National Program Component(s)?

The research relates to National Program 107, Human Nutrition (100%) and the nutrient requirements component of the program. Priority objectives addressed by the research are biomarkers, mechanism of action, nutrient interactions, environmental factors, and function and performance. This research will develop information about the effects of zinc, copper and selenium deficiencies on biochemical functions that will facilitate the detection of marginal deficiencies of these elements and define their dietary requirements for health, development and optimal performance throughout the life cycle. The research will also provide information that can be used to assess the risk of chronic disease from subclinical zinc, copper and selenium deficiencies.

4. What were the most significant accomplishments this past year?

A. Single Most Significant Accomplishment during FY 2000:

Pathological consequences of copper deficiency may arise from increased production of damaging oxygen radicals produced by the mitochondria as a result of decreased cytochrome c oxidase activity. Hydrogen peroxide production and activities of respiratory complexes I thru IV of the electron transport chain were assayed in mitochondria isolated from the livers of rats fed copper-deficient or control diets. Copper deficiency, as expected, lowered the activity of copper-dependent cytochrome c oxidase, and also significantly lowered the activity of the copper-independent respiratory complex I causing an increase in hydrogen peroxide production when electrons entered the electron transport chain at complex I. This work shows that copper deficiency, by inhibiting the initial step in mitochondrial electron transport, increases the production of reactive oxygen species and identifies mitochondrial dysfunction as a factor that increases the risk for chronic, degenerative diseases related to poor copper status.

B. Other Significant Accomplishments(s), if any:

Selenium has beneficial and toxic effects that may be perturbed by its interaction with other dietary or environmental trace elements. The effect of the interaction between selenium and arsenic on the programmed cell death signaling pathway was examined in human leukemia cells challenged with selenium or with selenium plus arsenic. Selenium caused

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necrosis in the cells but this effect was neutralized by the presence of arsenic. This finding indicates that the biological effects of selenium can be antagonized by arsenic at the cellular level and suggests that the establishment of daily selenium requirements depend on arsenic and other selenium-antagonistic elements found in diets.

5. Describe the major accomplishments over the life of the project, including their predicted or actual impact.

The effect of marginal copper deficiency during pregnancy on the expression of the alpha, beta, and gamma isoforms of protein kinase C in neonatal rat brain was examined. Copper deficiency reduced the rate of expression of the protein kinase C isoforms during the three weeks following birth and led to significant reductions of protein kinase C beta in the hypothalamus and protein kinase C gamma in the hypothalamus and cerebellum. Impact: Protein kinase C expression is a determinant of brain development. Impairment in the expression of protein kinase C isoforms may eventually explain how neurological function and intellectual development are affected in the offspring of mothers who are subclinically copper deficient during pregnancy and the perinatal period.

High concentrations of zinc in the diet, equal to two to three times the RDA that can be found in over-the-counter mineral supplements, can lower the copper status of humans and may eventually lead to signs of copper deficiency. It is hypothesized that this effect is caused by alterations in the transport of copper through the intestinal epithelial cell that is carried out by specific copper transport proteins, Menkes (MNK) and hCTR1. To study this phenomenon, a cell culture system was devised that uses an intestinal cell mimic derived from a human colon carcinoma cell (Caco-2), that when differentiated, has similar characteristics as an intestinal epithelial cell in the body. As predicted, moderate to high concentrations of zinc in the growth media of the cells inhibited copper transport. Western blotting analysis with a specific antibody to MNK showed that at least one transcript of the protein was reduced by moderate and high zinc concentration. In addition, the high amount of zinc in the media reduced the relative abundance of MNK mRNA, the gene product necessary for the production of the protein. Impact: This work strongly suggests that zinc affects the abundance of the two newly discovered copper transport proteins, MNK and hCTR1, and, for the first time, suggests a plausible mechanism for the initiation of low copper status in humans and animals fed excess zinc in their diets.

The body has some mechanism that tells it how much zinc it needs and tries to adjust accordingly. However, how this mechanism works is not known. We used an intestinal cell culture model to determine if different concentrations of zinc in the media regulate the rate of uptake and transport of zinc. We found that when cells were grown in media containing either 5 or 25 micromolar zinc, the rate of uptake into the

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cells and transport across a monolayer from the lumenal side to the basolateral side of cells was much lower with 25 micromolar than with 5 micromolar. However, in the reverse direction, the rate of uptake and transport was much higher. Impact: This finding shows for the first time that the cells are using two mechanisms to control how much zinc the body gets; 1) the potential for transport into the body is slowed, and 2) the potential for transport out of the body is increased. This adaption helps maintain a balance of zinc so that the body does not get overloaded. Recently, another laboratory discovered a zinc transporter protein that is thought to help transport zinc. We suggest that high zinc is down regulating the production of this transporter either at the level of transcription of the gene or at the translation step for production of the protein. Positive outcomes would give clues to the question that has intrigued the nutrition research community for decades; how does zinc regulate its own rate of absorption?

Copper deficiency increases the activities of manganese superoxide dismutase in liver and heart mitochondria and glutathione peroxidase in heart mitochondria. This finding demonstrates that mitochondria experience increased oxidative stress during copper deficiency. Furthermore, the increase in oxidative stress was accompanied by increased oxidative modifications of mitochondrial proteins. This finding indicates that even though manganese superoxide dismutase and glutathione peroxidase activities increased during copper deficiency, these compensatory increases in antioxidant protection were not sufficient to protect mitochondria from damage by oxygen radicals. Impact: Oxidative damage to mitochondrial components and associated mitochondrial dysfunction is a major factor that contributes to the development of degenerative diseases and ageing. This work identifies mitochondrial damage as a component of the biological outcomes of copper deficiency that could lead to increased risk for chronic, degenerative diseases related to poor copper status.

6. What do you expect to accomplish, year by year, over the next 3 years?

FY2001-02: The effects of copper deficiency on the production of reactive oxygen species by liver, heart and muscle mitochondria will be examined. These experiments will test the hypothesis that low copper status is a determinant of oxygen radical production by mitochondria and that mitochondria are the main source of reactive oxygen species that cause intracellular damage during copper deficiency. The actual mitochondrial site for the generation of reactive oxygen species will be determined. It also will be determined if the altered redox state of cells during copper deficiency induces the gene expression of proteins that respond to oxidative stress such as manganese superoxide dismutase and hemeoxygenase.

FY 2001-02: Studies to determine if maternal copper deficiency impacts fetal brain development by impairing either the genetic regulation of protein kinase C expression or intracellular distribution of protein

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National Program(s): 107 100%

kinase C will be completed.

FY 2001-02: Studies will be designed to further characterize the effect of zinc on the physiological function of the cell membrane transporters for copper. Zinc seems to affect the relative abundance of the MNK protein and its mRNA; however, it is not known exactly how zinc might have this effect. The question to be answered, is zinc involved in transcription factors that govern mRNA production, and/or is the efficiency mRNA translation affected by zinc? The answer to this question would significantly advance our knowledge about the regulation of transport of copper and about how cells regulate the accretion of trace elements in general.

FY 2001-02: Studies will be designed to further characterize the effect of zinc on the regulation of zinc transporter proteins. High zinc down regulates the rate of zinc uptake into and transport across intestinal cells. Is this because the transporter protein is not being manufactured or is the transporter itself being deactivated by its own substrate? Answers to these questions would significantly advance our knowledge about the regulation of transport of zinc and about how cells regulate the accretion and distribution of trace elements in general.

FY 2001-02: Studies will be designed to determine how zinc deficiency regulated the relative abundance of angiotensin converting enzyme mRNA in the testes. Zinc is intimately involved with certain transcription factors that regulate the transcription of mRNA. Are these factors affected by low dietary zinc concentration? Answers to these questions would significantly advance our knowledge about the regulation of gene transcription factors by dietary nutrients.

FY 2001-02: Studies will be designed to determine the mechanisms of how the concentration of dietary zinc regulates food intake. State-of-the-art feeding monitors will be used to study feeding characteristics in zinc deprived rats and the patterns correlated with bioactive peptides that seem to be regulated by zinc dependent enzymes. These include neuropeptide Y and cholecystokinin. Food intake is very responsive to dietary zinc intake, and is a good model to study food intake regulation in general.

FY 2001-02: The effect of selenium on cell cycle, and inhibition of apoptosis induced by other trace elements such as arsenic will be examined in a cell culture system.

FY 2001-02: Gene transcriptional regulation of copper/zinc superoxide dismutase and manganese superoxide dismutase in response to nutritional mineral trace elements will be examined with a gene reporter system. The results of the study will shed light upon the roles of mineral trace elements in gene transcriptional regulation of anti-oxidative enzymes.

FY2001-02: The effects of marginal deficiencies of calcium, zinc, and iron on cadmium availability from wheat products will be examined. Pasta will be the first wheat product studied in the series. The minimal concentrations of food calcium and iron that provide a protective effect against excessive cadmium absorption will be determined.

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FY2002-03: Investigations will be conducted to determine whether calcium, zinc and iron content in food crops can be altered to either take up less cadmium and/or to increase their concentration of calcium and iron in order to provide a natural deterrent to cadmium absorption by the consumer but, at the same time, maintain their natural nutritional value.

FY2002-03: Selenium related-genes will be identified that have anti-apoptotic properties and influence the cell cycle. Information from the investigation will advance our knowledge about the importance of selenium at molecular level when cells are under a stress condition.

FY 2002-03: Investigations will be initiated to determine if oxygen radicals generated within mitochondria during copper deficiency can cause permanent mitochondrial dysfunction by inducing mutations in mitochondrial DNA. It will be determined if different types of dietary carbohydrates, lipids, and antioxidants can potentiate or ameliorate oxidative damage caused by copper deficiency.

FY 2002-03: Studies will be initiated that will determine the involvement of zinc with the regulation of the activity of insulin in the regulation of growth and development. There has been a long association between zinc and insulin activity, but the mechanisms have not been worked out. Insulin degrading enzyme (IDE) is a zinc dependent enzyme and its substrates bind to transmembrane receptors and initiate a variety of biological effects. An enzyme that regulates the removal of these substrates could have extraordinary effects on cell growth and development. This may be one of the major roles of zinc in growth and development.

7. What science and/or technologies have been transferred and to whom? When is the science and/or technology likely to become available to the end user (industry farmer, other scientists)? What are the constraints, if known, to the adoption and durability of the technology products

Results from studies are published in peer-reviewed scientific journals. Upon publication, the methodology, data, and interpretations of the data are immediately available to scientists and other interested parties. In addition, experimental results and educational information pertinent to public interests are made available through the local newspaper, The Grand Forks Herald, and nationally and internationally through the Center's Web Site on the Internet. During FY2000, the following information was transferred to the public through articles published in the Grand Forks Herald: "Science: The Hidden Influence on Nutrition" by W.T. Johnson and "The ABCs of Good Nutrition" by Philip G. Reeves.

Data pertaining to cadmium concentrations in tissues and body fluids from human and animal studies were presented to consumer organizations interested in food additives for their consideration in setting recommendations for allowable cadmium concentrations in foods.

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Accession: 0401450 Year: 00 Project Number: 5450-51000-023-00 D
Mode Code: 5450-20-00 STP Codes: 5.1.3.3 80% 5.1.3.4 20%
National Program(s): 107 100%

8. List your most important publicatons in the popular press (no abstracts) and presentations to nonscientific organizations and articles written about your work (NOTE: this does not replace your peer-reviewed publications which are listed below)

None.

9. Scientific Publications:

01. Johnson, W.T., Thomas, A.C., Lozano, A.A. Maternal copper deficiency impairs the developmental expression of protein kinase C alpha, beta and gamma isoforms in neonatal rat brain. Nutritional Neuroscience. 2000. v. 3. p. 113-122.

02. Johnson, W.T., Prohaska, J.R. Gender influences the effect of perinatal copper deficiency on cerebellar PKC gamma content. Biofactors. 2000. v. 11. p. 163-169.

03. Johnson W.T. Copper deficiency does not increase hepatic mitochondrial hydrogen peroxide production. The FASEB Journal. 2000. v. 14. Abstract p. A773.

04. Reeves, P.G., DeMars, L.C., Briske-Anderson, M. Physiologic concentrations of media zinc alter the relative abundance of ATP7a mRNA and protein in CACO-2 cells. The FASEB Journal. 2000. v. 14. Abstract p. A228.

05. Momcilovic, B., Reeves, P.G. The effect of idiorrhythmic zinc dose-rate feeding on the induction of intestinal metallothionein. The FASEB Journal. 2000. v. 14. Abstract p. A512.

06. Johnson, W.T. "Copper deficiency does not increase hepatic mitochondrial hydrogen peroxide production" presented at the Experimental Biology 2000 meeting, San Diego, CA, April 2000.

07. Reeves, P.G. "Physiologic concentrations of media zinc alter the relative abundance of ATP7a mRNA and protein in CACO-2 cells presented at the Experimental Biology 2000 meeting, San Diego, CA, April 2000.

08. Reeves, P.G. "The effect of idiorrhythmic zinc dose-rate feeding on the induction of intestinal metallothionein" presented at the Experimental Biology 2000 meeting, San Diego, CA, April 2000.

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Title: CENTER DIRECTOR

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ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0403340 Year: 00 Project Number: 5450-51000-023-01 T
Mode Code: 5450-20-00 STP Codes: 5.1.3.3 50% 5.1.3.4 50%
National Program(s): 107 100%

Title: CORRELATION BETWEEN SPERM MOTILITY AND HEAVY METAL STATUS OF ANIMALS AND HUMANS

Period Covered From: 10/99 To: 09/00

Would you like to terminate this Project? N

Will this project terminate within the first 2 months of FY2001? N

Progress and Outcomes:

1. What major problem or issue is being resolved and how are you resolving it?

In the equine breeding industry, cryopreservation of the semen has not been perfected to the point where the freeze-thaw cycle does not cause low viability and sperm count, and/or low sperm motility. This results in reduced ability of the sperm to fertilize the egg. Human male fertility studies have shown that the trace element content of the semen, especially zinc, is an important factor that helps control sperm count, viability, and motility. We question whether this could be part of the cause, if not the sole cause, of similar problems in horses. In addition, could other trace elements at either essential and/or toxic concentrations, be part of the causative factor?

The initial phase of this study will be to collect body fluids such as semen, plasma, and urine from a population of stallions and measure the trace element content to ascertain the physiological status of each element. Human samples also will be collected. We will determine if there is a correlation between low to high concentrations of the elements and sperm viability, motility, and survival time during cryopreservation. The trace elements to be analyzed include calcium, zinc, copper, iron, selenium, cadmium, chromium, and others that might be of interest.

2. How serious is the problem? Why does it matter?

As new reproductive technologies have become available to the horse breeding industry, it has become apparent that there are unexpected problems affecting semen preservation and sperm viability. At present there is no good way to ship frozen semen to maintain high sperm viability, thus causing a major loss of the product at great cost to the breeders. Some of these problems could be related to the mineral content of the semen. There are viability problems with human sperm as well that could have a similar etiology.

3. How does it relate to the National Program(s) and National Program Component(s)?

This research will develop information about the effects of various

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National Program(s): 107 100%

mineral elements on the viability, motility, and biochemical functions of animal and human sperm. In addition, this research will allow us to determine safe and adequate intakes of mineral elements for optimal health and bodily functions. Thus, the research relates to National Program 107, Human Nutrition (100%) and the nutrient requirements component of the program. Priority objectives addressed by the research are biomarkers, mechanism of action, nutrient interactions, environmental factors, and function and performance.

4. What were the most significant accomplishments this past year?

A. Single Most Significant Accomplishment during FY2000:
None. This project has just begun.

B. Other Significant Accomplishments:
None.

5. Describe the major accomplishments over the life of the project, including their predicted or actual impact.

None. This project has just begun.

6. What do you expect to accomplish, year by year, over the next 3 years?

This project was initially set up only for a two year duration (FY 2001-2002). During this period, we will analyze numerous urine, semen, and blood samples from humans and horses to determine relationships between mineral content and in vitro function of mineral elements.

7. What science and/or technologies have been transferred and to whom? When is the science and/or technology likely to become available to the end user (industry farmer, other scientists)? What are the constraints, if known, to the adoption and durability of the technology products

None.

8. List your most important publicatons in the popular press (no abstracts) and presentations to nonscientific organizations and articles written about your work (NOTE: this does not replace your peer-reviewed publications which are listed below)

None.

9. Scientific Publications:
01.

Approved: FORREST H NIELSEN Date: 09/00
Title: CENTER DIRECTOR

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Accession: 0147990 Year: 00 Project Number: 5450-51000-023-03 T
Mode Code: 5450-20-00 STP Codes: 5.1.3.2 70% 5.2.2.2 30%
National Program(s): 107 100%

Title: HEALTH EFFECTS AND BIOAVAILABILITY OF CADMIUM FROM SUNFLOWER SEED KERNELS:A HUMAN STUDY

Period Covered From: 10/99 To: 09/00

Would you like to terminate this Project? Y

Will this project terminate within the first 2 months of FY2001? N

Progress and Outcomes:
1. What major problem or issue is being resolved and how are you resolving it?

A lack of sufficient knowledge about the cadmium content of food products, interactions between cadmium and other mineral elements, and absorption and retention of cadmium in the body results in the inability to reasonably establish intakes of cadmium from food that are toxic, and thus arise unreasonable trade restrictions against exports of food products from areas in which soil cadmium content is high or farming practices are such that cadmium uptake by plants into seeds used for food is promoted. This problem centers on the fact that some crops, including sunflowers, wheat, rice, and flax, that are grown in the upper Mid-Western United States contain a higher amount of cadmium (> 0.6 mg/kg) than similar crops grown in other parts of the country and the world. These producers are at an economic disadvantage in the global agricultural economy because of trade restrictions against importation of their products. Moreover, current international deliberations are proposing to further reduce the cadmium concentration allowed in imported foods. A clear description of the impact of food-borne cadmium from agricultural products, such as sunflower seeds, is needed to ascertain the biological impact of cadmium in foods on health indices.

Controlled studies of human volunteers were conducted to assess the absorption of cadmium into the body by feeding kernels containing a stable isotope of cadmium and determining the amount of unabsorbed labeled cadmium coming out in the feces. The difference between intake and output in the feces is a measure of absorption. Other studies involved the feeding of sunflower kernels containing cadmium for a long period (48 weeks) and determining if biological parameters that indicate accumulation of the cadmium in the body were affected. These parameters include cadmium concentrations in urine and red blood cells; increased concentrations would indicate that cadmium may be accumulating in the body and increasing the body burden.

Rats are fed diets containing graded amounts of iron, zinc and/or calcium to achieve a mild to moderate mineral deficiency state. They then are fed sunflower seed kernels that have been labeled with a radioisotope of cadmium and counted in an animal whole body counter to determine retention of the radioactive cadmium. The animals are sacrificed and various organs are collected and analyzed for cadmium and other minerals and blood biochemical indices of health. Measurements of cadmium

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National Program(s): 107 100%

retention provide a biological measure of the effect of marginal mineral deficiency on whole body cadmium burden and individual organ uptake of cadmium as indices of cadmium toxicity.

2. How serious is the problem? Why does it matter?

The seriousness of this problem is two-fold. Because cadmium is best known as a toxin, there is a perception that the intake of sunflower kernels containing a small amount of cadmium will increase the body burden of cadmium, and may adversely affect the health of an individual. A second concern is the assumption that the amount in the food, rather than the absorption and uptake of cadmium from food commodities, such as sunflower kernels, is a valid basis for trade restrictions of cadmium-containing food products into European markets and to warrant further restrictions and a possible loss of trade by US agricultural producers.

3. How does it relate to the National Program(s) and National Program Component(s)?

The research program relates to National Program 107, Human Nutrition, and emphasizes the Program Component Performance Goal 3.1.1 - Human Nutrition Requirements. It will determine the appropriate balance of nutrients and beneficial food components in the diet by using various in vivo and in vitro methods, ascertain the gastrointestinal metabolism, absorption and distribution of cadmium from foods, and identify new biomarkers of dietary cadmium intake and utilization. This research will allow us to establish safe and adequate intakes of cadmium for optimal health and bodily functions.

4. What were the most significant accomplishments this past year?

Cadmium is a trace element found in some foods that if consumed in large amounts can be detrimental to health. Other mineral elements in the diet are known to affect cadmium absorption, thus in collaboration with Dr. Rufus Chaney, USDA, ARS Environmental Chemistry Laboratory, Beltsville, MD, experiments were performed to determine if marginal dietary deficiencies of iron, zinc, and/or calcium affect the absorption and body burden of cadmium from sunflower kernels. The results showed that: (a) mild deprivations of iron and/or calcium significantly increase the amount of cadmium absorbed, and the amount deposited in various tissues, (b) moderate reductions in dietary zinc did not affect cadmium absorption, and (c) there was an interaction between iron and calcium such that the combined marginal deficiencies caused an even greater absorption and tissue accumulation of cadmium indicating that individuals consuming diets low in iron and calcium are more likely to accumulate more cadmium in their bodies than those consuming an adequate diet. These data, which were submitted to the FAO/WHO Joint Expert Committee on Food Additives

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(June 2000), show that cadmium toxicity during low level cadmium consumption is very dependent on the mineral nutrient status of an individual; consideration of mineral nutrition status is important when making decisions about allowable amounts of cadmium in food commodities.

5. Describe the major accomplishments over the life of the project, including their predicted or actual impact.

Although cadmium is viewed as a toxic element, there is a conspicuous lack of information about the effect of prolonged consumption of controlled amounts of cadmium on biological function and health. Human volunteers consumed cadmium-containing sunflower kernels for 48 weeks; their health was assessed with non-invasive and relevant biological tests. The data show that cadmium status indicators were not affected by feeding the cadmium-containing sunflower kernels for this long period. The information gathered in this study was forwarded to the Joint FAO/WHO Expert Committee on Food Additives (JECFA) meeting for presentation in June 2000; it will provide the Committee with useful information to use to develop a more rational approach to decision making about the allowable amounts of cadmium in sunflower kernels as well as other food commodities.

Animal studies show that cadmium from a diet containing 20% sunflower kernels is less available than from one containing no kernels, and studies with humans who report a regular consumption of more than 1 ounce of kernels/week show that they are more likely to have higher cadmium intakes than those who report eating less than one ounce/week; no adverse health effects were found as a result of consuming the high or higher amounts of sunflower kernels. With the support of the International Lead/Zinc Research Organization, Inc., and the National Sunflower Association (approximately $32,000 and $5,000, respectively) and USDA, ARS, NPS ($140,000), two human studies to assess the availability of cadmium from sunflower kernels on a long-term basis, and to test the effects of long-term controlled feeding of kernels were conducted. The findings indicated that the intestinal absorption of cadmium from the kernels ranges from 2 to 10%, but that no increase in body burden or adverse health effects when subjects consumed up to 9 ounces of kernels/wk for 48 weeks. The information gathered from our studies were used by the Joint FAO/WHO Expert Committee on Food Additives (June 2000) in discussions recommending limits on the amount of cadmium in certain food stuffs, including sunflower kernels and will provide a more rational approach to decision making about the allowable amounts of cadmium in sunflower kernels as well as other food commodities.

6. What do you expect to accomplish, year by year, over the next 3 years?

The funds for this trust have been depleted. This trust will be terminated. Scientific publications are in preparation or have been

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National Program(s): 107 100%

submitted for publication. These publications will be listed in the parent CWU 5450-51000-023-00D when published.

7. What science and/or technologies have been transferred and to whom? When is the science and/or technology likely to become available to the end user (industry farmer, other scientists)? What are the constraints, if known, to the adoption and durability of the technology products

The findings brought forth in this research have been presented to the Food and Drug Administration, the agricultural industry, other scientists, and have been published in scientific journals. All information is immediately available for adoption. In addition, some of the findings have been disseminated in the form of presentations at national and international scientific meetings. This year Dr. Philip Reeves attended the 31st Session of the Codex Committee on Food Additives and Contaminants, at The Hague, The Netherlands, for informal discussions of these findings with a host of international participants. All the data pertaining to cadmium concentrations in tissues and body fluids from human and animal studies were presented to the WHO Joint Expert Committee on Food Additives, Geneva, Switzerland, for their consideration in setting recommendations for allowable cadmium concentrations in foods. Dr. Reeves presented "The effects of trace mineral status on food cadmium absorption and tissue distribution in humans and animals" at the Scientific Committee on Problems of the Environment (SCOPE) Workshop on Environmental Cadmium in the Food Chain: Sources, Pathways, and Risks in Brussels, Belgium, September 2000.

8. List your most important publicatons in the popular press (no abstracts) and presentations to nonscientific organizations and articles written about your work (NOTE: this does not replace your peer-reviewed publications which are listed below)

As a result of this research, Dr. Reeves was invited to review the progress of the research at numerous meetings of the National Sunflower Association. In addition, this work was presented to the Grand Forks Human Nutrition Research Center's Focus Group, which was made up of members from many disciplines, including commodity groups, local business groups, city and university governancies, and representatives from the federal government.

9. Scientific Publications:

01. Reeves, P.G. Mineral nutrient status affects cadmium bioavailability from sunflower kernels. Proceedings of the 22nd Sunflower Research Workshop. 2000. p. 28-37.

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National Program(s): 107 100%

Publications: (Continued)

02. Reeves, P.G. Chaney, R.L. Nutrient status affects the absorption of cadmium from sunflower kernels. The FASEB Journal. 2000. v. 14. Abstract p. A750.

03. Reeves, P.G. "Nutrient status affects the absorption of cadmium from sunflower kernels" presented at the Experimental Biology 2000 meeting, San Diego, CA, April 2000.

Approved: FORREST H NIELSEN Date: 09/00
Title: CENTER DIRECTOR

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ANNUAL RESEARCH PROGRESS REPORT
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Accession: 0149978 Year: 00 Project Number: 5450-51520-011-00 D
Mode Code: 5450-20-00 STP Codes: 5.1.3.3 75% 5.2.2.2 25%
National Program(s): 107 100%

Title: BIOCHEMICAL, PHYSIOLOGICAL, AND NUTRITIONAL ROLES OF CERTAIN ULTRATRACE ELEMENTS

Period Covered From: 10/99 To: 09/00

Would you like to terminate this Project? N

Will this project terminate within the first 2 months of FY2001? N

Progress and Outcomes:
1. What major problem or issue is being resolved and how are you resolving it?

Although emerging evidence indicates that certain mineral elements (e.g., arsenic, boron, nickel, silicon and vanadium) often called ultratrace elements can be involved in the prevention or amelioration of disease with nutritional roots, or for the enhancement of health and longevity, insufficient evidence exists to develop credible and data-supported dietary recommendations for these elements to assure health and well-being. Moreover, inadequate knowledge about health benefits of some ultratrace elements that are now only being discovered or defined (e.g., boron in bone and joint health) results in inappropriate and ineffectual reliance on other mineral nutrients (e.g., calcium) to provide these benefits. The lack of appropriate dietary recommendations for ultratrace elements allows charlatans for the purpose of financial gain to inappropriately promote these elements as supplements that can prevent some feared diseases such as cancer, osteoporosis, heart disease, and loss of cognitive function, or can enhance physical appearance. Thus, some of these elements are promoted (e.g., vanadium) in such a way that intakes detrimental to health may be occurring. Finally, the lack of recognition of health benefits provided by some ultratrace elements gives rise to risk assessments and toxicological standards by regulatory agencies that conflict with amounts that are known or predicted to be beneficial to health; this results in unnecessary efforts and expenditure of funds to reduce environmental exposure to amounts that in reality are not harmful, and may actually have health benefits. In summary, defined biochemical functions for ultratrace elements such as arsenic, boron, nickel, silicon and vanadium will establish these elements as essential nutrients, will allow the development of status indicators for the determination of dietary requirements to prevent any decline in health and well-being, and will allow for appropriate risk assessments and toxicological standards.

Animal and human experiments are and will be conducted to define the biochemical and physiological roles of various

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ultratrace elements including arsenic, boron, nickel, silicon and vanadium. The basic approach is to feed experimental animals and human volunteers diets that contain low, adequate, and/or luxuriant amounts of specific ultratrace elements and other selected nutrients and non-nutrients (postulated to affect the metabolism and utilization of specific ultratrace elements). The response of the animals and humans to the dietary manipulations will be ascertained by evaluating appropriate biochemical, physiological and anatomical variables. Biochemical and molecular biology methods will be used to define the specific essential role(s) of various ultratrace elements.

2. How serious is the problem? Why does it matter?

Dietary factors are associated with 5 of the 10 leading causes of death and with numerous chronic disorders; these include coronary heart disease, certain types of cancer, stroke, atherosclerosis, hypertension, osteoporosis and arthritis. Among those diseases that are linked strongly to diet, the cost of treatment and care in the United States exceeds $200 billion per year. Recognition that nutrition is important in health promotion and disease prevention has spawned a plethora of "health-enhancing foods" and supplements, now often called "functional foods" or "nutraceuticals" that represent an exploding market in the United States which exceeds $29 billion per year. Many of the health claims for these functional foods and nutraceuticals, however, have not been substantiated by basic research and feeding trials. Many of the health claims include the use of ultratrace elements because of some promising physiological or clinical finding (most often in an animal model or a special human situation) that has been extrapolated to their intakes having an influence on the susceptibility to, or severity of, one or more chronic diseases. Thus, there is a need to establish which foods, and their amounts, that will provide appropriate quantities of specific ultratrace elements of practical importance for the promotion of health and disease prevention or alleviation. Additionally, there is a need to determine safe intakes of specific ultratrace elements so that the setting of reasonable toxicological standards can be accomplished. Fulfilling these needs should result in policies and programs that will result in a healthier population, decrease the burden of chronic disease, enhance the quality of life, and diminish health care and environmental exposure protection expenditures.

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3. How does it relate to the National Program(s) and National Program Component(s)?

The research program relates to the National Program 107, Human Nutrition, and emphasizes the Program Component Performance Goal 3.1.1 - Human Nutrition Requirements. The challenge of this component is to identify essential nutrients, determine their effects on reproduction, development, function and longevity, and to provide information that will be used to develop standards to optimize human health, well-being, and genetic potential throughout the life cycle. All priority objectives, especially mechanism of action, biomarkers, function and performance, and nutrient interactions apply to the research program. Outcomes of the research will be knowledge that will facilitate the detection and prevention of biochemical, structural, physiological and psychological dysfunctions caused by the deficiency or imbalance of specific ultratrace elements, and will define requirements and safe intakes of specific ultratrace elements for health and well-being throughout the life cycle.

4. What were the most significant accomplishments this past year?

A. Although other research groups have provided experimental evidence that boron is essential for the normal embryological development of lower animal species (i.e., frog and zebrafish), the importance of boron for embryological development of mammals needs to be established because this is more likely to be applicable to humans. Dietary boron, fed in nutritional amounts, changed the maturation rate of developing spinal column in an animal nutrition-pregnancy model. This is the first report indicating that boron has a role in the earliest stages of bone formation in mammals. This finding, along with the observation that boron interacts with erythritiol (a boron-binding substance) to affect fetal absorptions in the pregnancy model, suggest that boron may be important in embryo development in humans and supports the concept that boron is an essential nutrient for humans.

B. Selenium is recognized as being able to decrease the risk for certain types of cancer, but there is only limited knowledge about how selenium affects the susceptibility to chronic disease including cancer and heart disease. In a study examining how the lack of selenium increases the risk to certain types of cancer perform in collaboration with other Grand Forks Human Nutrition Research Center scientists, plasma

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and tissue homocysteine concentrations were measured because homocysteine has been identified as an independent risk factor for atherosclerotic disease. In the range from deficient to adequate selenium, the amounts of homocysteine in plasma, liver and heart were found to be directly proportional to dietary selenium. The finding shows that selenium may influence cardiovascular health through an effect on homocysteine metabolism.

In high concentrations, arsenic causes hypomethylation of DNA which is associated with an increased incidence of certain forms of cancer. In collaboration with other Grand Forks Human Nutrition Research Center scientists the dose response of arsenic on DNA methylation was determined in cells cultured on media containing negligible amounts of arsenic. When compared to cells cultured in media containing small amounts of arsenic, cells cultured in media containing negligible amounts of arsenic hypomethylated DNA. This finding demonstrates that there are beneficial amounts of arsenic that can decrease the risk to certain forms of cancer, in addition to toxicological amounts that can increase that risk, and that toxicological standards need to consider that both too little and too much arsenic can have detrimental consequences.

5. Describe the major accomplishments over the life of the project, including their predicted or actual impact.

In an arthritic animal model, physiological amounts of dietary boron increased concentrations of natural killer cells and also attenuated the rate of paw swelling. These findings indicate that nutritional amounts of boron reduce the risk to inflammatory disease by helping hold in check a system that is constantly poised to attack, a balance that permits pathogen elimination but avoids autoimmunity. Showing that boron controls the severity of a form of experimental rheumatoid arthritis suggests that a nutritionally adequate intake of boron could have an impact on the occurrence and severity of this disease which has a national cost similar to that for heart disease and stroke.

Vanadium was found to be a nutritionally important element involved in thyroid hormone function and glucose metabolism. The amount needed for this involvement was extremely small, and thus indicated that supranutritional amounts, similar to those provided by supplements available over-the-counter to the public, could induce changes that could be construed as not beneficial. This suggests that some over-the-counter supplements and formulas sold as anabolic or anti-diabetic

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agents could be detrimental to health.

Vanadium status affected the response of rats to N-nitro-L-arginine methyl ester (L-NAME), an inhibitor of nitric oxide (an important reactive oxygen species) formation, and oxidative metabolism status indicators. The findings suggest that vanadium has a biological role involving a reactive oxygen species and supports the concept that vanadium is an essential nutrient but needed in very low amounts.

Arsenic deprivation was shown to be important in methionine metabolism apparently by affecting methionine recycling. Two metabolites of methionine were altered by arsenic deprivation in rats; liver S-adenosylhomocysteine (SAH) was increased while S-adenosylmethionine (SAM) was decreased which resulted in a decreased ration of SAM to SAH. A low SAM/SAH ratio has been associated with an increased risk for certain types of cancer; thus, although high arsenic apparently can increase risk to certain types of cancer, the findings indicate that too low of intakes of arsenic can also increase the risk to certain types of cancer. This possibility should be considered by groups setting toxicological standards for arsenic because standards set too low could result in unnecessary expenditure of funds to reduce oral intakes to amounts that could actually have unfavorable health effects.

Arsenic was found to be a nutritionally beneficial ultratrace element whose effects are modified by stressors of sulfur amino acid metabolism (e.g., methionine). Among the enzymes involved in this metabolism whose activity was altered by arsenic when stressors were present was the liver enzyme betaine-homocysteine methyltransferase. This indicates that arsenic has a nutritional role involving biological methylation and support the concept that arsenic is an essential nutrient.

An interaction between arsenic and copper showed that high dietary arsenic can exacerbate signs of copper deficiency. Extremely high arsenic (compared to normal nutritional intakes) had adverse effects on ceruloplasmin, heart size and the concentration of mineral elements in liver and kidney in rats fed low dietary copper. Thus, pathological conditions such as cardiovascular disease attributed to arsenic toxicity may in reality be caused by copper deficiency. Increasing copper intakes may be the most efficient and cost-effective mechanism of decreasing the incidence of heart disease associated with elevated amounts of arsenic in drinking water.

By the use of the isotope nickel-63, it was found that 2.5% of nickel ingested by rats is absorbed and very little is retained. A model suggesting that nickel is homeostatically controlled by absorption and retention processes was

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developed. In this model, nickel is metabolized by at least three different mechanisms. The model and findings provide further evidence that nickel is an essential element for higher animals and humans, and thus could be of nutritional importance.

Novel mathematical approaches (logistic regression, discriminant analysis) were developed that can be used to derive more objective-based dietary guidance and can be easily adapted to new information as it becomes available. Because the models account for interactive effects (synergistic or antagonistic), they can be tailored to a specific group of individuals. The mathematical approaches also have given an improved method for assessing the status of specific nutrients which uses mathematically combined parameters rather than individual parameters; the combined parameters give a better correlation to the status of the nutrient in question. More accurately determining the need for mineral nutrients will promote the intakes necessary for optimal health and well-being.

6. What do you expect to accomplish, year by year, over the next 3 years?

FY 2001 - The effects of boron deprivation and physiological amounts of dietary boron on rheumatoid arthritis using human volunteers will be determined with an emphasis on measuring variables that will indicate whether low boron nutriture, not uncommon in the United States, is a factor in the prevalence and severity of rheumatoid arthritis.

Mathematical models will be extended and refined to show the importance of interactions among trace elements in determining their dietary requirements.

By the use of isotopic arsenic, attempts will be made to isolate arsenic-dependent proteins from animals; the relationship of these isolated proteins to methyl metabolism will be determined.

Animal and cell culture experiments will be done to determine the effect of graded concentrations of arsenic on DNA methylation to test the hypothesis that compared to physiological or nutritional amounts of arsenic, the lack of arsenic, or toxic amounts of arsenic, will induce hypomethylation which is associated with a greater susceptibility to some forms of cancer.

A diet low in silicon will be developed that can be fed to human volunteers so it can be established that silicon is a nutritionally important element.

FY 2002 - Studies will be initiated to elucidate the biochemical mechanism whereby dietary boron affects blood

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National Program(s): 107 100%

clotting regulation.

Animal experiments will be conducted to ascertain whether nickel has a function at the cell membrane level affecting signal transduction involving ionized calcium and/or phosphate moieties. Gene arrays will be used to establish whether nickel up or down regulates specific genes involved in membrane signaling, membrane channels, and nitric oxide metabolism.

Evidence will be obtained that will establish whether arsenic deprivation affects the susceptibility to certain types of cancer. The experiments will involve animals fed diets either containing negligible or nutritional amounts of arsenic and with half of the rats from each dietary group given a substance that induces cancer.

Specific tissues from animals that have been fed diets lacking or adequate in arsenic will be used in gene arrays to determine whether or not specific genes related to methionine metabolism are up or down regulated (turned on or off); differential changes will be used to establish the role of arsenic in methionine and methyl metabolism.

FY 2003 - Studies will be initiated to elucidate the mechanism through which boron induces increases in natural killer cells in the serum of animal models with experimental rheumatoid arthritis.

An experiment with human volunteers will be initiated to establish whether they respond to nickel deprivation in a manner similar to animals and thus establish nickel as nutritionally important. Status indicators developed from animal experiments in FY 2001 and FY 2002 will be used.

An experiment with human volunteers will be initiated to establish whether silicon is needed for healthy connective tissue function, including wound healing.

7. What science and/or technologies have been transferred and to whom? When is the science and/or technology likely to become available to the end user (industry farmer, other scientists)? What are the constraints, if known, to the adoption and durability of the technology products

Information about the nutritional or beneficial aspects of ultratrace elements as it becomes available is routinely transferred to a variety of customers. The customers include risk assessments groups through direct contact or organized meetings and workshops; the public through web pages of professional organizations and the Grand Forks Human Nutrition Research Center, and via popular media; and other scientists through presentations at national and international meetings and professional publications.

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Technological advances and concerns involved in the estimation of dietary boron intakes of the U.S. population were transferred to nutrition scientists and industry representatives at a formal discussion held in conjunction with a national scientific meeting in San Diego, CA, April 17, 2000.

Information was transferred to the public through articles in the local newspaper (Grand Forks Herald) which also were placed on the Grand Forks Human Nutrition Research Center Home Page; these articles were "Gleaning Nutrient Content" and "Health Research Requires Volunteers" by Curtiss Hunt, and "Arsenic in Water: What's Safe" by Eric Uthus.

8. List your most important publicatons in the popular press (no abstracts) and presentations to nonscientific organizations and articles written about your work (NOTE: this does not replace your peer-reviewed publications which are listed below)

Articles in the popular media often mention boron as a nutrient important for bone and joint health; this is based on findings from research done in this CWU. No attempt is made to keep a record of these articles.

9. Scientific Publications:

01. Hunt, C.D. Dietary boron is a physiological regulator of the normal inflammatory response. In: Roussel, A.M., Favier, A.E., Anderson, R.A. (eds.). Trace Elements in Man and Animals - TEMA 10. Plenum Publishing, New York, NY. 2000. p. 1071-1076.

02. Nielsen, F.H. The dogged path to acceptance of boron as a nutritionally important mineral element. In: Roussel, A.M., Favier, A.F., Anderson, R.A. (eds.). Trace Elements in Man and Animals - TEMA 10. Plenum Publishing, New York, NY. 2000. p. 1043-1047.

03. Nielsen, F.H. The ultratrace elements. In: Stipanuk, M.H. (ed.). Biochemical and Physiological Aspects of Human Nutrition. W.B. Saunders, Philadelphia, PA. 2000. p. 825-840.

04. Uthus, E.O., Gao, J., Finley, J.W., Davis, C.D. and Nielsen, F.H. Selenium status affects arsenic deprivation in rats. In: Centeno, J.A., Collery, P., Vernet, G., Finkelman, R.B., Gill, H. and Etienne, J.-C. (eds.). Metal Ions, v. 6. John Libbey Eurotext, Montrouge, France. 2000. p. 254-256.

05. Keehr, K.A. and Hunt, C.D. Dietary boron and erythritol affect reproduction and fetal development in rats. Proceedings of the North Dakota Academy of Science. 2000. v. 54. p. 35.

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National Program(s): 107 100%

Publications: (Continued)

06. Hunt, C.D. Apparent beneficial effects of dietary boron on rat reproduction are modified by dietary erythritol. FASEB Journal. 2000. v. 14. p. A478.

07. Uthus, E.O., Finley, J.W. and Davis, C.D. Selenium deprivation decreases total plasma homocysteine in rats fed a torula yeast-based diet. FASEB Journal. 2000. v. 14. p. A536.

08. Zaslavsky, B., Milne, D.B., Nielsen, F.H. and Uthus, E.O. Statistical assessment of copper status in postmenopausal women. FASEB Journal. 2000. v. 14. p. A221.

09. Armstrong, T.A., Spears, J.W., Flowers, W.L. and Nielsen, F.H. Boron affects growth and serum metabolites, but not reproductive characteristics in swine. FASEB Journal. 2000. v. 14. p. A478.

10. Uthus, E.O., Gao, J., Finley, J.W., Davis, C.D. and Nielsen, F.H. Selenium status affects arsenic deprivation in rats. In: 6th International Symposium on Metal Ions in Biology and Medicine, Scientific Program and Book of Abstracts. San Juan, Puerto Rico. 2000. p. 133.

11. Nielsen, F.H., Uthus, E.O., Gao, J. and Yokoi, K. Malapropos risk assessment of some supposedly hazardous mineral elements could have detrimental economic and health consequences. In: 32nd Great Lakes 2000 American Chemical Society Regional Meeting Abstracts of Presentations. Fargo, ND. 2000. p. 17.

12. Hunt, C.D. "Apparent Beneficial Effects of Dietary Boron on Rat Reproduction are Modified by Dietary Erythritol" presented at the Experimental Biology 2000 meeting, San Diego, CA, April 2000.

13. Uthus, E.O. "Selenium deprivation decreases total plasma homocysteine in rats fed a torula yeast-based diet" presented at the Experimental Biology 2000 meeting, San Diego, CA, April 2000.

14. Zaslavsky, B. "Statistical assessment of copper status in postmenopausal women" presented at the Experimental Biology 2000 meeting, San Diego, CA, April 2000.

15. Uthus, E.O. "Selenium status affects arsenic deprivation in rats" presented at the Sixth International Symposium on Metal Ions in Biology and Medicine, San Juan, Puerto Rico, May 2000.

16. Nielsen, F.H. "Malapropos assessment of some supposedly hazardous mineral elements could have detrimental economic and health consequences" presented at the 32nd Great Lakes 2000 American Chemical Society Regional Meeting, Fargo, ND, June 2000.

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Publications: (Continued)
17. Hunt, C.D. "Boron: A New Trace Element in Human Health" presented to the University of North Dakota Department of Anatomy and Cell Biology, Grand Forks, ND, April 3, 2000.

Approved: FORREST H NIELSEN Date: 09/00
Title: CENTER DIRECTOR

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Accession: 0401535 Year: 00 Project Number: 5450-51520-011-03 T
Mode Code: 5450-20-00 STP Codes: 5.1.3.3 50% 5.2.2.2 50%
National Program(s): 107 100%

Title: THE NUTRITIONAL ROLE OF BORON IN THE INHIBITION OF SERINE PROTEASES

Period Covered From: 10/99 To: 09/00

Would you like to terminate this Project? N

Will this project terminate within the first 2 months of FY2001? N

Progress and Outcomes:

1. What major problem or issue is being resolved and how are you resolving it?

The cause of rheumatoid arthritis is unknown, a cure is unavailable, and the lifetime cost of rheumatoid arthritis for an individual patient can be as much as $250,000. Thus, research is needed to find the cause of the disease and more effective treatments. Excessive inflammation leads to inflammatory disease (for example, rheumatoid arthritis). Certain boron compounds are potent in vitro inhibitors of several enzymes that regulate the normal inflammatory reaction. Therefore, the focus of this project is to identify inflammatory mediators that interact with boron. This approach will help establish the specific function of boron in humans and how dietary boron influences rheumatoid arthritis. Based on molecular structure, several classes of compounds predicted to interact with boron will be selected and assessed to determine the exact role of boron in regulation of the inflammatory response. The basic approach will be to determine the in vitro binding affinities of these compounds to boron. Subsequently, compounds with physiologically significant boron binding affinities will be investigated as potential indicators of boron status by in vivo measurement of these compounds after extraction from tissues of animals fed boron deficient diets.

2. How serious is the problem? Why does it matter?

Inflammatory diseases cost Americans billions of dollars yearly in treatment and loss of productivity. Rheumatoid arthritis in particular is a painful, chronic, recurrent, systemic inflammatory disease that affects 1-3 percent of Americans. Thus, prevention or significant amelioration of inflammatory diseases including rheumatoid arthritis by relatively simple dietary means, would have significant impact. There is a high probability that normal amounts of dietary boron will significantly ameliorate symptoms of rheumatoid arthritis in humans based on its known effect on cartilage development and maintenance and influence on the progression of experimental rheumatoid arthritis in animal model systems. Identification of biomolecules that interact directly with boron will serve to define borderline boron deficiency and develop indicators of boron deficiency.

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3. How does it relate to the National Program(s) and National Program Component(s)?

The research relates to the National Program 107, Human Nutrition (100%), specifically, the National Program Component of Human Nutrition Requirements: "determine requirements for nutrients and other food components of children, pregnant and lactating women, adults, and elderly of diverse racial and ethnic backgrounds." The research relates to the "Mechanism of Action" objective within this component: "Identify and fully characterize mechanisms of action for beneficial effects of known nutrients and other potentially beneficial dietary chemicals; measure the size of the effects associated with specific amounts of the chemical or nutrient component in question."

4. What were the most significant accomplishments this past year?

Identification of biomolecules that interact directly with boron will serve to define borderline boron deficiency and develop indicators of boron deficiency. A new capillary electrophoresis method developed earlier in this laboratory was used to demonstrate the direct binding of boron to biomolecules of considerable physiological importance. Normal physiological amounts of boron were found to bind to a family of cell signal hormones, the diadenosine poly-phosphates, that controls cell growth and the response of the cell to physiologic stress, two factors important in the inflammatory process. These findings suggest that boron, a natural dietary component, may be important in the regulation of cell growth.

5. Describe the major accomplishments over the life of the project, including their predicted or actual impact.

An in vitro model system was developed and implemented to determine the direct binding of boron to biomolecules. The method utilizes capillary electrophoresis technology and allows for the discrete separation and identification of biomolecules that bind to boron and also allows for discrimination of binding interactions. When molecules are separated by capillary electrophoresis in the absence or presence of boron, the degree of boron binding is indicated as a change in migration time of the molecule through the solution. An increase in migration time indicates increased boron binding. Various biomolecules can be compared for their ability to bind boron. The information may be used to determine the importance of the exact biological role of boron in humans and the importance of that role in the inflammatory response. The finding that cells isolated from a mouse monocyte-macrophage cell line and grown in a normal culture medium internalize boron provides additional indirect

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National Program(s): 107 100%

evidence for an essential role for boron in animals and humans.

6. What do you expect to accomplish, year by year, over the next 3 years?

FY 2001: New methodology will be developed and implemented to identify the large number of biomolecules, isolated from whole blood, that are predicted to bind to physiological amounts of boron. FY 2002: Experiments will be conducted to determine whether tissue concentrations of biomolecules that bind to boron and are known to have a role in inflammation are influenced by boron-deprivation in an experimental rheumatoid arthritis animal model. FY 2003: Experiments will be conducted to determine the physiologic amount of dietary boron needed to provide maximal amelioration of experimental rheumatoid arthritis and optimal blood concentrations of biomolecules previously determined to bind to boron.

7. What science and/or technologies have been transferred and to whom? When is the science and/or technology likely to become available to the end user (industry farmer, other scientists)? What are the constraints, if known, to the adoption and durability of the technology products

The capillary electrophoresis method for determining and evaluating the interaction between boron and biomolecules was described and discussed to nutrition scientists and industry representatives at a formal boron discussion held at the same time and location of a national scientific research meeting, San Diego, CA, April 17, 2000.

8. List your most important publicatons in the popular press (no abstracts) and presentations to nonscientific organizations and articles written about your work (NOTE: this does not replace your peer-reviewed publications which are listed below)

None.

9. Scientific Publications:

01. Ralston, N.V.C., Hunt, C.D. Biological boron interactions: charge and structure characteristics required for boroester formation with biomolecules. The FASEB Journal. 2000. v. 14(4). Abstract p. A538.

02. Ralston, N.V.C., Hunt, C.D. Boron incorporation by RAW 264.7 macrophages as indicated by differences in intracellular and extracellular boron concentrations. The FASEB Journal. 2000. v. 14 (4). Abstract p. A530.

03. Ralston, N.V.C. "Biological boron interactions: charge and structure characteristics required for boroester formation with biomolecules" presented at the Experimental Biology 2000 meeting, San Diego, CA, April 2000.

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Report of Progress (AD-421)

Accession: 0401535 Year: 00 Project Number: 5450-51520-011-03 T
Mode Code: 5450-20-00 STP Codes: 5.1.3.3 50% 5.2.2.2 50%
National Program(s): 107 100%

Publications: (Continued)

04. Ralston, N.V.C. "Boron incorporation by RAW 264.7 macrophages as indicated by differences in intracellular and extracellular boron concentrations" presented at the Experimental Biology 2000 meeting, San Diego, CA, April 2000.

Approved: FORREST H NIELSEN Date: 09/00
Title: CENTER DIRECTOR

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ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0402077 Year: 00 Project Number: 5450-51520-011-04 T
Mode Code: 5450-20-00 STP Codes: 5.1.3.3 80% 5.2.2.2 20%
National Program(s): 107 100%

Title: DETERMINATION OF THE ESSENTIALITY OF NICKEL

Period Covered From: 10/99 To: 09/00

Would you like to terminate this Project? N

Will this project terminate within the first 2 months of FY2001? N

Progress and Outcomes:

1. What major problem or issue is being resolved and how are you resolving it?

The lack of a defined biochemical function for nickel in higher animals has prevented the determination of the nutritional importance of this element which has strong circumstantial evidence indicating that it is an essential nutrient. Based on defined biochemical functions for nickel in lower forms of life, nickel most likely has a beneficial or essential action(s) needed for health and well-being of humans. This action or function of nickel needs to be identified.

Experiments will be conducted with rats and possibly chicks to identify metabolic pathways that are influenced by dietary nickel. Initially, these experiments will be factorially arranged with low and adequate amounts of nickel fed in diets formulated to alter certain specific pathways by the inclusion of nutritional stressors. Once a pathway influenced by nickel is identified, biochemical and molecular biological methods will be used to define the exact locus where nickel is functioning. Knowing this function will allow the determination of a status indicator. If a status indicator is identified, the response of human volunteers to a nickel low diet will be ascertained.

2. How serious is the problem? Why does it matter?

Lack of unequivocal acceptance of nickel essentiality inhibits the consideration of possible health consequences when regulatory agencies or groups develop risk assessments and toxicological standards for nickel that might conflict with amounts people should consume for health benefits. Unnecessary conservative assessments and standards for safe intakes of nickel could cause economic burdens for the public through the expenditures in resources to reduce exposure to amounts that are not harmful. Moreover, the lack of a status indicator for nickel inhibits the determination of the

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National Program(s): 107 100%

nutritional importance, and prevents the provision of sound dietary guidance for this element, which animal findings suggest is important in the nitric oxide-cGMP signal transduction system which is important for olfaction (smell), vision, and some cardiovascular functions. Thus, defining a biochemical function for nickel in higher animals and humans, which would establish nickel as an essential nutrient, would be of economic benefit to the U.S. populace, public utilities and industry, and most likely would result in dietary guidance that would enhance the health and well-being of the public.

3. How does it relate to the National Program(s) and National Program Component(s)?

The research fits into the National Program 107, Human Nutrition (100%), and emphasizes the Program Component Performance Goal 3.1.1 - Human Nutrition Requirements. The challenge of this Component is to identify essential nutrients, determine their effects on reproduction, development, function and longevity, and to provide information that will be used to develop standards to optimize human health, well-being, and genetic potential throughout the life cycle. All priority objectives, especially mechanism of action, biomarkers, function and performance, and nutrient interactions apply to the research program. Outcomes of the research will be knowledge that will facilitate the detection and prevention of biochemical, structural, physiological and psychological dysfunctions caused by the deficiency of nickel, and will define the requirement and safe intake of nickel for health and well-being throughout the life cycle.

4. What were the most significant accomplishments this past year?

A. The biochemical function, and thus the nutritional importance, of nickel still are unclear but recent findings suggest that nickel has a biochemical function associated with vitamin B6 (pyridoxine), vitamin B12, and folic acid, vitamins whose deficiencies have been associated with increased circulating homocysteine, a new risk factor for cardiovascular disease. An experiment was performed in which rats were fed diets low and adequate in nickel, and contained elevated amounts of homocystine (oxidized form of homocysteine) or methionine (precursor of homocysteine) and deficient pyridoxine as possible nutritional stressors of nickel function. Dietary nickel deficiency increased plasma pyridoxal phosphate without changing urinary 4-pyridoxic acid excretion, and increased urinary nitrate excretion without

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National Program(s): 107 100%

changing urinary cyclic GMP excretion. The findings indicate that nickel has a biochemical function that affects pyridoxine metabolism and the nitric oxide-cGMP signal transduction system in mammals, and thus is important in processes such as olfaction (smell) and vision.

B. Biological impedance spectroscopy (BIS) is a versatile method for assessing body water compartments and other bioelectric properties in humans, but it has not been applied to experimental animals. In collaboration with Grand Forks Human Nutrition Research Center scientists, BIS and Bioelectric Impedance Analysis were used to ascertain whether nickel status affected possible changes in body composition and cell membrane integrity induced by aberrant homocysteine status. Excessive dietary sulfur amino acids (i.e., homocystine and methionine) decreased whole body capacitance corrected for body length and intracellular water; this change was not affected by nickel status, but nickel deprivation increased fat mass without changing fat-free mass. The findings indicate that BIS is as versatile in experimental animals as in humans; excessive dietary sulfur amino acids causes cellular membrane damage; and nickel has a function that affects energy metabolism.

Because epidemiological evidence indicates an association between high circulating homocysteine and renal disorders, and animal findings indicate that nickel status affects the response to excessive dietary homocysteine, a study was performed to ascertain whether nickel status alters the detrimental effects associated with an increased body content of homocysteine. Rats were fed diets containing low and adequate dietary nickel and normal and excessive dietary homocystine (oxidized form of homocysteine); variables indicating renal damage were determined. Excessive dietary homocystine increased circulating homocysteine, and induced signs of renal damage including increased cell numbers (hyperplasia), blood in the urine, and increased urinary excretion of albumin and phosphate; nickel did not markedly affect the renal changes. The findings indicate that high circulating homocysteine may be a cause, and not the result, of renal damage, and that nickel does not have a function that influences this toxicological effect of homocysteine.

5. Describe the major accomplishments over the life of the project, including their predicted or actual impact.

All major accomplishments are listed above (Question #4).

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6. What do you expect to accomplish, year by year, over the next 3 years?

Funds for this project have been provided for 2 years only; the project is in its second year.

FY 2001 - Animal experiments will be performed to determine whether nickel has a role in the nitric oxide-cGMP signal transduction system. Experimental animals (rats) will be fed nickel deficient or supplemented diets; some will be also fed L-NAME, a nitric oxide synthase inhibitor. The nitric oxide-cGMP signal transduction system affects vascular smooth muscle relaxation, and central nervous system function. Thus, several aspects of behavior will be tested, including auditory startle response, and behavior tests for smell and vision, in addition to biochemical tests to ascertain changes in cGMP amounts and actions.

FY 2002 - 2003 - If this project continues, and a nickel function is identified that can be related to a blood parameter, a preliminary study, followed by a complete study using preliminary data, both using human volunteers will be planned and conducted to ascertain whether the parameter varies with nickel intakes that commonly occur and whether the variation has any practical nutritional implications.

7. What science and/or technologies have been transferred and to whom? When is the science and/or technology likely to become available to the end user (industry farmer, other scientists)? What are the constraints, if known, to the adoption and durability of the technology products

Information about the nickel essentiality and its practical nutritional importance as it becomes available is routinely transferred to a variety of customers. The customers include risk assessments groups through direct contact or organized meetings and workshops; the public through web pages of professional organizations and the Grand Forks Human Nutrition Research Center, and via the popular media; and other scientists through presentations at national and international meetings and professional publications.

8. List your most important publicatons in the popular press (no abstracts) and presentations to nonscientific organizations and articles written about your work (NOTE: this does not replace your peer-reviewed publications which are listed below)

None

9. Scientific Publications:

01. 1. Nielsen, F.H., Yokoi, K. and Uthus, E.O. Marginal dietary pyridoxine and supplemental dietary homocystine and methionine affect the response of the rat to nickel deprivation. In: Centeno, J.A., Collery, P., Vernet, G., Finkelman, R.B., Gill, H. and Etienne, J.-C. (eds.). Metal Ions, v.6. John Libbey Eurotext, Montrouge, France. 2000. p. 524-527.

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Accession: 0402077 Year: 00 Project Number: 5450-51520-011-04 T
Mode Code: 5450-20-00 STP Codes: 5.1.3.3 80% 5.2.2.2 20%
National Program(s): 107 100%

Publications: (Continued)

02. 2. Yokoi, K. Hall, C.B., Lukaski, H.C., Uthus, E.O. and Nielsen, F.H. Estimation of body water by bioimpedance spectroscopy (BIS) in rats fed methionine or homocystine. FASEB Journal. 2000. v. 14. p. A497.

03. 3. Yokoi, K., Uthus, E.O. and Nielsen, F.H. Dietary homocystine induces renal damage in rats. FASEB Journal. 2000. v. 14. p. A796.

04. 4. Nielsen, F.H., Yokoi, K. and Uthus, E.O. Dietary nickel alters the response of rats to deficient dietary pyridoxine, and to high dietary homocystine or methionine. FASEB Journal. 2000. v. 14. p. A539.

05. 5. Nielsen, F.H., Yokoi, K. and Uthus, E.O. Marginal dietary pyridoxine and supplemental dietary homocystine affect the response of the rat to nickel deprivation. In: 6th International Symposium on Metal Ions in Biology and Medicine, Scientific Program and Book of Abstracts. San Juan, Puerto Rico. 2000. p. 202.

06. 6. Yokoi, K., Uthus, E.O. and Nielsen, F.H. Supplemental sulfur amino acids (SAA) affect pyridoxine metabolism. In: 32nd Great Lakes 2000 American Chemical Society Regional Meeting Abstracts of Presentations. Fargo, ND. 2000. p. 17.

07. 7. Yokoi, K. "Estimation of body water by bioimpedance spectroscopy (BIS) in rats fed methionine or homocystine" presented at the Experimental Biology 2000 meeting, San Diego, CA, April 17, 2000.

08. 8. Yokoi, K. "Dietary homocystine induces renal damage in rats" presented at the Experimental Biology 2000 meeting, San Diego, CA, April 18, 2000.

09. 9. Nielsen, F.H. "Dietary nickel alters the response of rats to deficient dietary pyridoxine, and to high dietary homocystine or methionine" presented at the Experimental Biology 2000 meeting, San Diego, CA, April 17, 2000.

10. 10. Nielsen, F. H. "Marginal dietary pyridoxine and supplemental dietary homocystine affect the response of the rat to nickel deprivation" presented at the Sixth International Symposium on Metal Ions in Biology and Medicine, San Juan, Puerto Rico, May 10, 2000.

11. 11. Yokoi, K. "Supplemental sulfur amino acids (SAA) affect pyridoxine metabolism" presented at the 32nd Great Lakes 2000 Regional Meeting of the American Chemical Society, Fargo, ND, June 5, 2000.

Approved: FORREST H NIELSEN Date: 09/00
Title: CENTER DIRECTOR

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Accession: 0400356 Year: 00 Project Number: 5450-51530-003-00 D
Mode Code: 5450-20-00 STP Codes: 5.1.3.3 75% 5.3.1.1 25%
National Program(s): 107 100%

Title: MINERAL ELEMENTS, PHYSIOLOGICAL FUNCTION & PERFORMANCE AND BODY COMPOSITION

Period Covered From: 10/99 To: 09/00

Would you like to terminate this Project? N

Will this project terminate within the first 2 months of FY2001? N

Progress and Outcomes:
1. What major problem or issue is being resolved and how are you resolving it?

Recommendations for the dietary intake of mineral elements, with an emphasis on copper, chromium, iron, magnesium, and zinc, based on the promotion of health and optimal biological function are generally lacking. Studies designed to examine the effects of graded dietary intakes of mineral elements on physiological function are needed to ascertain appropriate amounts of mineral elements in the diet to maintain health and to facilitate the attainment of genetic potential of biological functions. One consideration in delineating appropriate dietary mineral intakes is assessment of food-borne factors that affect the absorption and utilization of dietary minerals. Also, the use of environmental stressors (i.e., controlled exercise and cold/hot temperatures) is another factor used to determine mineral element needs of physically active people.

Iron deficiency is the single most prevalent nutritional deficiency in the world. Attempts to fortify food products have been only partially successful in ameliorating this pervasive nutritional problem among women. In parallel with the incidence of iron deficiency, there has been an increase of polyunsaturated fat intake. Studies indicate that the type of dietary fat affects iron absorption and utilization, specifically non-heme iron. Polyunsaturated fat reduces and saturated fat, specifically stearic acid, promotes iron utilization. Because stearic acid is neutral to serum cholesterol and lipoprotein cholesterol concentrations, it offers practical benefits in ameliorating iron deficiency in animals and humans.

Routine assessment of human body composition is hampered by the lack of sensitive and specific methods to measure bone mass and quality, fat and muscle in national nutritional surveys and repeatedly in response to medical and nutritional interventions.

Studies are conducted in animals and humans. Graded intakes of dietary zinc and copper are fed and physiological functions are monitored to delineate intake amounts that affect physiological function with an emphasis on energy utilization, cardiorespiratory function, work performance, and heat production. Studies of human volunteers fed whole food diets low in chromium and supplemented with specific chromium compounds and other compounds hypothesized to influence the absorption and utilization of chromium and other minerals are conducted. Other studies are undertaken in which animals are fed a diet low in iron, then given diets containing varied amounts of iron (low and adequate) and different

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National Program(s): 107 100%

types (saturated and polyunsaturated) of dietary fat. Changes in iron status and hematology, as well as changes in bone mineral content, are determined.

Studies are undertaken in animals and humans to develop and validate methods for use in assessing nutritional status and effects of nutritional intervention on bone and soft tissue composition.

2. How serious is the problem? Why does it matter?

There is considerable debate regarding the amount of dietary copper, chromium, magnesium, and zinc required for health maintenance and optimal biological function. Previous approaches focused on relatively insensitive measures of nutritional adequacy (chemical balance). By relating dietary mineral intakes to measurements of biological function, such as energy utilization, heart rate and blood pressure, work production, heat generation, and glucose and lipid metabolism, suggestions for dietary mineral intakes are made in reference to quality and quantity of life. Much of this research is requested by physically active individuals who seek to optimize physiological function without the use of dietary supplements and health managers who seek to minimize health care costs of the American public.

Development and validation of sensitive and specific methods to assess human body composition remain fundamental needs for nutritional assessment of healthy persons of all ages and backgrounds as well as individuals with chronic disease and medical intervention. Moreover, these methods are required for establishment of national distributions or norms for body fat and muscle for use as references in nutritional interventions to promote health.

3. How does it relate to the National Program(s) and National Program Component(s)?

This work relates to National Program 107, Human Nutrition(100%). The specific Program Components include Nutrient Requirements with the objectives of defining Biomarkers of Marginal or Borderline Deficiencies, Mechanisms of Action of Mineral Elements, Effects of Environmental and Lifestyle Factors, and Achievement of Optimal Function and Performance.

This research will acquire information about the effects of graded trace element deficiencies, emphasizing copper, chromium, iron, magnesium, and zinc, on biochemical measurements of mineral nutritional status and physiological functions. This information will facilitate the detection of marginal mineral deficiencies and define dietary requirements of these mineral elements for the development and maintenance of health and optimal function throughout the life cycle. The research will provide needed information that can be used to assess the risk of chronic diseases and impairments in subtle physiological functions that arise from mild and moderate mineral element deficiencies. In addition, new methods for

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National Program(s): 107 100%

routine assessment of human body composition will be developed and validated for non invasive assessment of human nutritional status, particularly conditions that exhibit weight change.

4. What were the most significant accomplishments this past year?

A. Single Most Significant Accomplishment During FY 2000:
Epidemiological surveys suggesting that the prevalence of obesity is increasing in the United States used only crude measurements of standing height and body weight; thus, more valid methods such as bioelectrical impedance are needed to measure body composition of a nationally representative sample of American adults and children to determine the validity of the suggestion. Investigators from the USDA, ARS Grand Forks Human Nutrition Research Center, St. Luke's-Roosevelt Hospital, Wright State University, and University of Chicago, with funding from the National Institutes of Health and the National Center for Health Statistics, developed and validated models to predict body composition based on measurements of bioelectrical impedance, then used impedance data from the Third National Health and Nutrition Examination Survey (NHANES III) in these models to determine the body composition of nationally representative samples of American children and adults. This study produced the first estimates of fat-free mass and body fat for non-Hispanic white, non-Hispanic black and Mexican Americans aged 12-80 years. These data provide an interim distribution of body composition parameters among three racial groups that will be useful in assessing future trends in fatness, a predictor of chronic disease, and fat-free mass, a key marker of sarcopenia, in the US population.

B. Other Significant Accomplishment:
Chromium is a mineral element for which a recommended dietary allowance (RDA) has not been established. Healthy premenopausal women were randomized into treatment groups receiving a placebo, chromium picolinate (200 micrograms chromium)and picolinic acid and fed a whole-food diet containing an average of 35 micrograms of chromium for 12 weeks. Compared to placebo, neither chromium nor picolinic acid supplementation had any effect on body composition or nutritional status indicators. These findings indicate that chromium supplementation does not promote weight loss, favorable changes in body composition or impair nutritional status of other minerals; apparently 35 micrograms of chromium meets the needs of premenopausal women.

5. Describe the major accomplishments over the life of the project, including their predicted or actual impact.

Simple, non-invasive methods, which are accurate and reliably determine changes, to assess human nutritional status, particularly muscle mass, are

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lacking. We adapted the tetrapolar bioelectrical impedance method, that was developed at the Grand Forks Human Nutrition Research Center, for the assessment of muscle mass in the upper legs (thighs) of overweight women who participated in a controlled weight loss program. Results showed that the bioimpedance determinations of muscle were similar to the reference measurements made with dual x-ray absorptiometry. Impact: These initial findings indicate that this new method is a valid and accurate method for routine determination of muscle mass in humans; this impedance approach also was a better indicator of muscle mass than the currently used measures of thigh circumference to estimate muscle mass.

Assessment of zinc nutritional status is complicated by the limited availability of blood biochemical markers that clearly distinguish between adequate and deficient zinc status and differentiate between degrees of zinc status, marginal and severe deficiency states. We evaluated the response of a zinc-containing enzyme in plasma, extracellular superoxide dismutase, in a series of studies of rats fed different amounts of dietary zinc and zinc-deficient rats supplemented with zinc. We found that the activity of this enzyme was more responsive to differences in dietary zinc, particularly in the rehabilitation of the zinc-deficient rats, than the currently used biochemical indicators of zinc status, including plasma zinc. Impact: These findings suggest that extracellular zinc superoxide dismutase activity is a more specific marker of zinc status than currently available indicators of zinc status, such as plasma zinc concentration, and that it may be useful in assessing human zinc nutritional status among individuals in national nutritional surveys.

Restricted dietary zinc adversely affected energy utilization during exercise in young men. As compared to a dietary zinc intake of 18 mg/d, 3 mg zinc daily was associated with significant alterations in energy production and respiratory function during progressive peak exercise on a cycle ergometer. The mechanism of this impairment was a significant decrease in red blood cell carbonic anhydrase activity with restricted dietary zinc. Carbonic anhydrase is a zinc-containing enzyme with the specific function of transporting carbon dioxide from cells to the lungs for excretion. Zinc deficiency was confirmed with a significant loss of zinc, negative zinc balance, and decreased serum zinc concentration when dietary zinc was restricted. Impact: These findings provide the first evidence of impaired physiological function when dietary zinc is fed in an amount similar to that consumed by some physically active individuals. Also, the finding of decreased carbonic anhydrase activity in the red blood cell in response to low dietary zinc suggests that the activity of this zinc-containing enzyme may be a new blood biochemical marker for assessment of human zinc nutritional status.

Blood cells are not an appropriate tissue for determination of chromium nutritional status. A pilot study revealed that the chromium concentrations of populations of white blood cells and platelets were too low (i.e., similar to background) to be useful as diagnostic measures of human chromium status. Although efforts to reduce background contamination

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were successful, the very low concentrations of chromium in these cells were difficult to measure accurately. Impact: The static measurement of cellular chromium is inadequate as a routine determination of human chromium nutritional status.

Restriction of dietary copper resulted in altered temperature regulatory function of rats acutely exposed to cold air. Copper deficiency was associated with an increased rate of loss of body temperature, decreased enzymatic conversion of the active form of thyroid hormone, triiodothyronine, from thyroxine, and decreased activity of the rate limiting enzyme dopamine beta hydroxylase, a copper-containing protein, needed for increased production of norepinephrine, the key regulator of heat production. A key finding was the identification of decreased transcription and translation of uncoupling protein that is required for heat production and thermogenesis in brown adipose tissue. The lack of induction of uncoupling protein formation is caused by a depressed expression of the genetic message for a specific heat shock protein, HSP 70, in copper deficiency. Importantly, the adverse effects of copper deprivation are ameliorated with copper repletion within three days of copper supplementation. Impact: These findings provide important information explaining the mechanism through which copper regulates energy metabolism, and explain why humans with an inborn inability to absorb copper suffer from hypothermia despite adequate hemoglobin concentration.

Supplementation of men with chromium picolinate while participating in controlled resistance training failed to demonstrate increased strength gain, facilitate loss of body fat and enhance muscle mass accretion. Impact: The results of this controlled study served as the basis for the Federal Trade Commission to rule that claims of propitious effects of chromium picolinate supplementation were without scientific basis. Similarly, the U.S. Pharmacopeia has concluded that chromium picolinate does not promote weight loss, facilitate body fat loss or promote muscle mass gain.

Consumption of dietary magnesium in amounts generally consumed by U.S. women resulted in alterations in energy production during submaximal exercise. Postmenopausal women fed 150 mg of magnesium daily demonstrated an increased oxygen consumption and elevated heart rates during submaximal exercise on a cycle ergometer as compared to a diet providing 350 mg magnesium daily, the recommended dietary intake. Magnesium deficiency was documented with increased losses of magnesium and altered blood ionized magnesium, that is consistent with increased mobilization of magnesium from bone, and decreased skeletal muscle magnesium. Impact: These findings provide the first evidence of diet-induced magnesium deficiency in otherwise healthy adults, and demonstrate that dietary magnesium intake consistent with amounts generally consumed by a majority of U.S. women are inadequate to support moderate intensity physical activities of daily life. Because significant physiological impairments were found at magnesium intakes consistent with national estimates of usual intake, there is a need for increased public health education to bolster dietary

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magnesium intakes to maintain physiological function and health.

Stearic acid promotes non-heme iron absorption and utilization in iron-deficient animals. Studies in rats and canines repeatedly showed that stearic acid enhanced the uptake and transfer of non-heme iron from the intestinal mucosa and increased red blood cell volume and hemoglobin concentration. This beneficial effect occurred at high (30%) and moderate (20%) intakes of stearic acid. Furthermore, the enhancement of iron metabolism with stearic acid did not adversely impact calcium or magnesium status. Impact: Stearic acid may be the uncharacterized component of meat, the "meat factor", that promotes non-heme iron utilization. Although saturated fatty acids generally increase atherogenic risk by increasing serum cholesterol and low density lipoprotein cholesterol concentrations, stearic acid has no adverse effects on cholesterol or lipoproteins. Use of stearic acid in recipes containing non-meat foods may reduce the incidence of iron deficiency anemia in humans.

6. What do you expect to accomplish, year by year, over the next 3 years?

FY 2001: Develop, validate and implement a new technical method for the use of tetrapolar bioelectrical impedance analysis for the assessment of regional muscle mass in humans. A model will be developed in weight-stable adults for determination of thigh muscle mass by using magnetic resonance imaging as a reference method. This model will be evaluated in adults participating in resistance training to increase muscle mass. This study will determine the advantages of specific impedance instruments (i.e., single or multiple frequency) in the predictive accuracy and sensitivity of muscle and fat determinations. If this approach is successful, a practical method will be available for generalized use in the routine assessment of human nutritional status in national surveys and hospital use with patients gaining or losing muscle and fat mass.

FY 2001: Determine the individual and combined effects of graded dietary zinc and copper on activities of superoxide dismutase enzymes as markers of zinc and copper status relative to traditional biochemical markers of zinc and copper nutriture of rats. Incorporate measures of extracelluar superoxide dismutase protein to assess the specific activity of this enzyme as a marker of zinc status and evaluate if expression of message of this protein is affected by dietary minerals. Findings will provide a basis for planning future human studies to delineate mineral requirement to control oxidative damage during physical training.

FY 2001: Ascertain the effects of graded dietary copper on resting energy metabolism and thermogenesis in the cold in rats. Test the hypothesis that copper restriction impairs the induction and expression of uncoupling proteins. Determine the effects of copper depletion independently of anemia on the metabolic perturbation in energy metabolism of copper-deficient rats. This study will identify new metabolic role(s) of copper in regulating energy expenditure.

FY2001: Delineate the effects of dietary zinc and exercise on the

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induction and expression of zinc-containing enzymes involved in energy production in rodents. By using graded dietary zinc and different types of exercise training (endurance and resistance), this study will determine if limiting zinc intake has adverse effects on ability to improve physical performance by limiting the production and activity of specific zinc-containing enzymes. Findings will provide a practical basis for assessing zinc requirements of physically active persons.

FY 2002: Determine the effect of type and amount of fatty acids on the transport and uptake of iron and other mineral elements by intestinal cells. Determine the induction and expression of cellular transport proteins of Caco-2 cells in vitro to elucidate the molecular mechanisms of dietary fat on trace element absorption. Results will define the molecular mechanisms of action of different fatty acids on iron uptake and transport and will compliment findings from studies of animals.

FY2002: Plan and initiate a health and nutrition survey of Native American tribes in North Dakota to determine relationships among mineral nutritional status and health with an emphasis on obesity, diabetes and cardiovascular disease. Results will provide a basis for planning future mineral supplementation trials to test the hypothesis that remediation of mineral deficiency will decrease incidence of some chronic diseases.

FY2002: Plan and initiate studies in humans to determine the effects of altered fatty acid composition of oilseeds and meat on circulating lipids and lipoproteins and hence risk of chronic disease. Results will be used to support large trials of fatty acid modification in grains and meat to promote human health and enhance value added nature of certain agricultural products.

FY2003: Determine the effects of increased zinc intake, either as food fortification or as supplement, on growth and body composition of adolescents with varying zinc status. Findings will be used to support food fortification strategies to optimize growth and function in a group that has an increased need for zinc because adolescence is a period of rapid growth and development.

FY2003: Design and implement a study of older adults to discern the impact of antihypertensive medications on zinc and magnesium status and assess the impact of supplemental zinc and magnesium, either as supplements or food fortification, on muscle function and body cell mass.

7. What science and/or technologies have been transferred and to whom? When is the science and/or technology likely to become available to the end user (industry farmer, other scientists)? What are the constraints, if known, to the adoption and durability of the technology products

A public presentation on the roles that dietary minerals play in the protection against development of coronary heart disease was made for the members of the Ontario Health Board. Information on the efficacy and safety of creatine, a popular nutritional supplement used by athletes, was transferred to the public through an article in the local newspaper, Grand

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Mode Code: 5450-20-00 STP Codes: 5.1.3.3 75% 5.3.1.1 25%
National Program(s): 107 100%

Forks Herald, "Safety Questions Should Temper Creatine Use", August 23, 2000. Information about the basic and applied aspects of the tetrapolar bioelectrical impedance method have been transferred to other federal agencies that are using this technology to continue to derive national body composition norms that will be made available to the public. Results from studies are published in peer-reviewed scientific journals. Upon publication, the methodology, data, and interpretations of the data are immediately available to scientists and other interested parties. In addition, experimental results and educational information pertinent to public interests are made available through the local newspaper, The Grand Forks Herald, and statewide through the Interactive Video Network of the North Dakota State University Service Continuing Education Program.

8. List your most important publicatons in the popular press (no abstracts) and presentations to nonscientific organizations and articles written about your work (NOTE: this does not replace your peer-reviewed publications which are listed below)

University of North Dakota, Department of Athletics, January 7, 2000, Grand Forks, ND. Lecture Alcohol, Sport and Performance.

9. Scientific Publications:

01. Lukaski, H.C. Chromium as a supplement. McCormick, D.B., Bier, D.M., Goodridge, A.G., Parmer, R., editors. Annual Reviews, Inc., Palo Alto, CA. Annual Review of Nutrition. 1999. v.19. p. 279-302.

02. Lukaski, H.C. Assessing regional muscle mass with segmental measurements of bioelectrical impedance in obese women during weight loss. Annals of the New York Academy of Sciences. 2000. v.904. p. 154-158.

03. Lukaski, H.C. Magnesium, zinc, and chromium nutriture and physical activity. American Journal of Clinical Nutrition. 2000. v.72(suppl). p. 585S-93S.

04. Ellis, K.J., Bell, S.J., Chertow, G.M., Chumlea, W.C., Know, T.A., Kotler, D.P., Lukaski, H.C., Schoeller, D.A. Bioelectrical impedance methods in clinical research: a follow up to the NIH technology assessment conference. Nutrition. 1999. v.15. p. 874-880.

05. Bolonchuk, W.W., Siders, W.A., Lykken, G.I., Lukaski, H.C. Association of dominant somatotype of men with body structure, function during exercise, and nutritional assessment. American Journal of Human Biology. 2000. v.12. p. 167-180.

06. McLaren, C.E., Kambour, E.L., McLachlan, G.J., Lukaski, H.C., Li, X., Brittenham, G.M., McLaren, G.D. Patient-specific analysis of sequential haematological data by multiple linear regression and mixture distribution modelling. Statistics in Medicine. 2000. v.19. p. 83-98.

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0400356 Year: 00 Project Number: 5450-51530-003-00 D
Mode Code: 5450-20-00 STP Codes: 5.1.3.3 75% 5.3.1.1 25%
National Program(s): 107 100%

Publications: (Continued)

07. Lukaski, H.C. Dietary fatty acids and minerals. Chow, C.K., editor. Marcel Dekker, Inc., New York, NY. Fatty Acids in Foods and Their Health Implications, 2nd Ed, Revised and Expanded. 2000. p. 541-555.

08. Lukaski, H.C., Siders, W.A., Hall, C.B. Evaluation of regional bioelectrical impedance measurements to assess whole body composition. Proceedings of the North Dakota Academy of Science. 2000. v.54. p. 38.

09. Siders, W.A., Lukaski, H.C., Hall, C.B. Adjustment to a peak physical work capacity treadmill test. Proceedings of the North Dakota Academy of Science. 2000. v.54. p. 41.

10. Lukaski, H.C., Hall, C.B., Siders, W.A. Assessment of regional muscle mass in obese women during weight loss with segmental measurements of bioelectrical impedance (Z). Fifth International Symposium on in vivo body compositions studies. 1999. Abstract.

11. Lukaski, H.C., Marchello, M.J., Hall, C.B., Siders, W.A. Validity of dual x-ray absorptiometry (DXA) to assess body composition of rats exposed to various stressors. The FASEB Journal. 2000. v.14. Abstract p. A486.

12. Lukaski, H.C., Vanderpool, R.A., Johnson, P.E. Decreased exchangeable copper after exercise in men fed diets varying in copper content. 32nd Great Lakes 2000 Regional Meeting American Chemical Society. 2000. Abstract p. 16.

13. Chumlea, W.C., Guo, S.S., Kuczmarski, R.j., Johnson, C.L., Flegel, K., Heymsfield, S., Lukaski, H., Schoeller, D., Friedl, K., Hubbard, S. Estimates of body composition from nationally representative NHANES III BIA data. The FASEB Journal. 2000. v.14. Abstract p. A39.

14. Guo, S.S., Chumlea, W.C., Heymsfield, S., Lukaski, H.C., Schoeller, D., Friedl, K., Kuczmarski, R.J., Flegel, K. Bioelectrical impedance (BIA) prediction equations for nationally representative NHANES III BIA data. The FASEB Journal. 2000. v.14. Abstract p. A39.

15. Lukaski, H.C. "Increased nutritional requirements for physically active people during aging" presented at the Northland Chapter, American College of Sports Medicine meeting, Grand Forks, ND, October 1, 1999.

16. Lukaski, H.C. "Validity of dual x-ray absorptiometry to assess body composition of rats exposed to various nutritional stressors" presented at the Experimental Biology 2000 meeting, San Diego, CA, April 2000.

17. Lukaski, H.C. "Evaluation of regional bioelectrical impedance measurements to assess whole body composition" presented at the Annual Meeting of the North Dakota Academy of Science, Fargo, ND, April 28-29, 2000.

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National Program(s): 107 100%

Publications: (Continued)

18. Lukaski, H.C. "Decreased exchangeable copper after exercise in men fed diets varying in copper content" presented at the 32nd Great Lakes 2000 Regional Meeting, American Chemical Society, Fargo, ND, June 4-6, 2000.

19. Lukaski, H.C. "Dietary zinc, carbonic anhydrase activities and human physical performance" presented at the 5th International Conference on the Carbonic Anhydrases, Port Townsend, WA, May 23, 2000.

Approved: FORREST H NIELSEN Date: 09/00
Title: CENTER DIRECTOR